MENGO ENCEPHALOMYELITIS VIRUS

THESIS

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The work presented in this Thesis was commenced at the Yellow Fever Research Institute, Entebbe, Uganda. It was continued at the International Health Division laboratory of the Rockefeller Foundation, New York, during tenure of a Rockefeller Foundation Fellowship, and additional studies were made while employed as Research Fellow in Epidemiology, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland.

"The animal kingdom offers intriguing possibilities and surprises".

Karl F. Meyer (1934)

"The deviation of man from the state in which he was originally placed by nature seems to have proved to him a prolific source of diseases. From the love of splendour, from the indulgences of luxury, and from his fondness for amusement, he has familiarized himself with a great number of animals which may not originally have been intended for his associates."

Edward Jenner (1796)

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INTRODUCTION

Twenty years ago the various human encephalitides then known, (excluding poliomyelitis and rabies), were listed by Rivers (1) as (a) Von Economo's lethargic, (b) following vaccination, (c) Japanese 1924, (d) Koritschoner and (e) Australian X disease. At that time from none of these diseases had a virus been recovered. In 1930 the viruses of louping ill (2) and Western equine encephalitis (3) were isolated. During the past two decades an additional 17 primary or suspected encephalitic viruses of man have been recovered.

Of these recently isolated agents the following have been recovered at the Yellow Fever Research Institute, Entebbe, Uganda: -West Nile (4), Bwamba Fever (5), Bunyamwera (6), Semliki Forest (7), Mengo encephalomyelitis (8), and Uganda S (9) viruses.

It is the purpose of this thesis to describe the isolation of Mengo encephalomyelitis virus and to discuss the properties of this agent and its relationship to the virus of encephalomyocarditis (10) and to the Columbia SK (11) and MM viruses (12).

A brief classification of the virus encephalitides of man will help to orientate the virus of Mengo encephalomyelitis, and the encephalomyocarditis, SK and MM viruses.

The encephalitides affecting man (of known virus etiology) may be grouped as follows:

a) The primary epidemic encephalitides which include

Western equine (3), Eastern equine (13), St. Louis (14), Japanese B (15), Russian Far Eastern (Spring-Summer) (16) and Venezuelan equine (17) encephalitis. With these should be included the various types of poliomyelitis virus, such as the Lansing (18) Y-SK (19) rodent adapted types and the Brunhilde and Leon (20) types of poliomyelitis virus. Jungeblut et al (11) are of the opinion that their strain of SK virus (now called Columbia-SK) and MM virus (21) are high titre mutants of poliomyelitis virus.

b) The primary non-epidemic encephalitides. In this group are the viruses of lymphocytic choriomeningitis (22), pseudolymphocytic choriomeningitis (23), Swineherd's disease (24), louping ill (2), encephalomyocarditis (10), and Mengo encephalomyelitis (8). The virus of rabies and Sabin's B. virus (25) may also be included in this group.

c) The suspected primary virus encephalitides which may or may not be epidemic in nature. Little, as yet, is known of the epidemiology of this group of agents, but humoral antibodies to each of them has been found in man. Included in this category are West Nile (4), Bwamba Fever (5), Bunyamwera (6), Semliki Forest (7), Hammon-Reeves California (26), Ilhéus (27) and Uganda S. (9) viruses.

d) The non-epidemic encephalitides which are produced by ordinarily non-encephalitogenic viruses, such as herpes simplex, mumps, measles, 17D yellow fever and lymphogranuloma venerum.

In the first place the history of the isolation of Mengo encephalomyelitis virus and a summary of the recovery of the encephalomyocarditis virus and the Columbia SK and MM viruses will be presented. The relationship of these 4 agents will be discussed. The pathogenicity of this group of viruses for animals and man will be described. Finally, the physical properties of this group, cultivation in the embryonated egg, and the pathological lesions induced in animals will be reported.

MENGO ENCEPHALOMYELITIS VIRUS

Mengo encephalomyelitis virus was so called because it was isolated in the Mengo district of Uganda, and be-

cause it can produce lesions in the brains and spinal cords of infected laboratory animals, and encephalitic symptoms in man.

ISOLATION OF VIRUS

Mengo encephalomyelitis (ME) virus was first isolated from a paralysed rhesus monkey which had not been subjected to any experimental procedures. Subsequent isolations were made from a mixed batch of mosquitoes of the genus Taeniorhynchus, from a batch of Taeniorhynchus (Coquillettidia) fuscopennatus Theobald mosquitoes, from a mongoose (Ichneumia sp.) and from a human. About a year after the first isolation, a 6th strain of the virus was isolated from another of the laboratory stock of unused rhesus monkeys. Evidence will be introduced to show that all these agents are strains of ME virus.

Isolation from a paralysed rhesus monkey

On June 19, 1946 a rhesus monkey (UR 22) from the reserve stock of monkeys was brought into the laboratory because it did not look well. The stock of monkeys from which UR 22 came had been kept in open-air runs in the compound of the Yellow Fever Research Institute, Entebbe, Uganda. Rhesus UR 22 had a normal temperature. Three days later it was found to have a flaccid paralysis of both legs and slight weakness of the right arm. Specimens of its blood and cerebro-spinal fluid were

taken and groups of mice[1] were inoculated intracerebrally with these materials. The monkey was then sacrificed. Areas of slight congestion were found in the lower lumbar region of its spinal cord, but apart from that no gross pathological changes were observed in any of the viscera. Portions of cortex, midbrain, pons, medulla, cerebellum, cervical, upper thoracic, lower thoracic and lumbo-sacral cord were taken for inoculation of mice and for histopathological examination. Each portion of brain and cord was ground separately in normal saline solution to make an approximately 10 per cent suspension[2]. These suspensions were centrifuged at low speed for 10 minutes and the supernates, both Seitz-filtered and unfiltered, were injected intracerebrally into groups of 6 adult mice and intraperitoneally into 4 mice 4 days old.

Mice inoculated with the blood serum of UR 22 and with suspensions of its cortex, pons, cerebellum and medulla remained well throughout an observation period of 30 days. Of the 6 mice inoculated with the cerebrospinal fluid of this monkey, 1 became paralysed on the 8th day and died on the 11th day after inoculation; the other mice of this group remained well. Among the 6 mice inoculated with the Seitz-filtered suspension from the midbrain, 1 became paralysed on the 6th day and died on the 8th day. Of the 6 adult mice inoculated with the unfiltered supernate of the suspension of the cervical and upper thoracic portions of the spinal cord, 2 were paralysed on the 4th day and sacrificed for sub-inoculation purposes and for preparation of dried mouse-brain virus; 1 mouse was paralysed on the 11th day and remained

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1. A group of mice consisted of 6 animals which, under routine, were caged together on weaning at 21 days of age. The mice employed in these experiments were descended from the original stock of Swiss white mice from Carworth Farms near New York City. At the time of use in experiments, mice were between 35 and 42 days old unless otherwise stated.
2. All suspensions and dilutions referred to in this paper are in terms of weight to volume and do not take into account loss by centrifugation, filtration or other procedures.

so until the 30th day, when it was destroyed. Of the 4 infant mice inoculated intraperitoneally with the latter suspension, 1 died on the 3rd and another on the 5th day, while the other 2 remained well. Of 6 adult mice inoculated with the Seitz-filtered supernate of the cervical and upper thoracic portions of the cord, 2 became paralysed on the 4th day and 1 on the 5th day; these were sacrificed for subinoculation purposes; one mouse of this group became paralysed on the 6th day and died on the 7th day, while another which became paralysed on the 6th day and 1 which became paralysed on the 8th day were still paralysed on the 30th day, when they were destroyed.

Of the 6 adult mice inoculated intracerebrally with the unfiltered supernate of the lower thoracic and lumbosacral portions of the cord, 1 became paralysed on the 3rd day, 1 was paralysed and 1 was sick on the 4th day and 2 were paralysed on the 5th day. All of these mice were sacrificed for subinoculation purposes or for the preparation of dried mouse-brain virus. The remaining mouse in this group became paralysed on the 6th day and died on the 11th day. All of the 4 infant mice inoculated intraperitoneally with this suspension were dead on the 3rd day. In the final group of adult mice inoculated intracerebrally with the Seitz-filtrate of this suspension, 1 mouse was paralysed on the 3rd day, 2 on the 5th day and 1 on the 6th day. All of these mice were sacrificed for subinoculation purposes or for preservation of mouse-brain virus; the other 2 mice of the group remained well for 30 days.

Successful intracerebral transfers were carried out with 10 per cent suspensions of the brains of paralysed adult mice which had been sacrificed and of dead baby mice. Three lines of mouse brain to brain passage were made. The "A" line originated from the brain of a mouse, paralysed on the 4th day, belonging to the group inoculated with a Seitz-filtered suspension of the cervical and upper thoracic cord of monkey UR 22; and the "B" and "C" lines originated from the brains of mice, paralysed on the 4th and 5th day respectively, from a group inoculated with an unfiltered suspension of the lower thoracic and lumbo-sacral cord of the same monkey. By the 2nd intracerebral passage there was 100 per cent mortality of the subin-

oculated groups of mice and the average survival time of inoculated mice was 4.0 days in each group. By the 7th intracerebral passage of mouse-brain virus the average survival times of mice in lines "A", "B", and "C" were 1.6, 2.2 and 2.8 days respectively. Routine transfers were continued with line "A".

The average survival time of mice inoculated intracerebrally from the 9th to the 13th transfer was 2 days, and for those inoculated from the 14th to 17th transfer it was 1.5 days. From the 18th to 29th passages, transfers of unfiltered brain suspension were made intraperitoneally. The average survival time of mice thus inoculated was 2.1 days from the 18th to 21st transfer, 1.8 days from the 22nd to 25th, and 2.0 days from the 26th to the 29th. Subsequently subinoculations were made intraperitoneally, using 0.06 ml. quantities of the Seitz-filtered supernate of 10 per cent mouse-brain virus. The average survival time in mice thus inoculated was 1.7 days from the 30th to the 39th transfer. Since the 40th transfer the average survival time has remained very constant at about 1.3 days. This strain of virus is now (June 1949) in its 108th passage in mice, having been maintained in refrigerated suspensions between mouse passages for various periods since June 1946.

The isolations of virus from the paralysed rhesus monkey, UR 22, were therefore from the cerebrospinal fluid and from various portions of the spinal cord.

Isolation from Taeniorhynchus mosquitoes.

Thirty-one days after UR 22 monkey was found paralysed a strain of virus was isolated from a group of mosquitoes consisting of the Taeniorhynchus species. The mosquitoes were from a batch of 444 mosquitoes of various species which were caught at Zika, a forested area about 7 miles from Entebbe. The mosquito catch was one of a series of many routine catches which form part of the yellow fever investigation programme in Uganda and

the method employed in making such catches has been described elsewhere (28). The mosquitoes were sorted (by Dr. A. J. Haddow) into groups convenient for inoculation of animals. In the Taeniorhynchus group there were 3 Taeniorhynchus (Coquillettidia) maculipennis Theobald, 96 T.(C.) fuscopennatus Theo., 19 T. (Mansonioides) africanus Theo., and 1 T. (M.) uniformis Theo.

After sorting, the mosquitoes were inactivated by chilling in a refrigerator and were then ground up with 3.0 ml. of physiological saline solution and centrifuged at low speed for 15 minutes. Mouse groups were inoculated with the unfiltered and Seitz-filtered supernates. One of the 6 mice inoculated with the unfiltered suspension was sick on the 9th day, but successful transfers were not made with the brain of this mouse. Of 6 mice inoculated with the Seitz-filtrate, 1 was found dead on the 12th day; all others remained well for 30 days. Transfers of mouse-brain suspensions were made from this dead mouse. Subinoculated groups of mice did not exhibit 100 per cent mortality till the 4th intracerebral passage. After the 5th transfer in 1 line of mouse-brain passage, mice were paralysed or dead within 24 hours, the average survival time by the 6th passage (with the use of unfiltered 10 per cent mouse-brain suspension) was 1 day. The average survival time of mice in passages 6 to 9 (in which Seitz-filtered 10 per cent mouse-brain virus was passed intracerebrally) was 1.1 days. From the 10th to the 15th passage the subinoculations were made by the intraperitoneal

route, and the average survival time was 1.9 days.

Thirty-five days after monkey UR 22 was found paralysed, an evening mosquito catch was made in and around the outdoor monkey run where UR 22 had been kept. The catch consisted of 216 Taeniorhynchus (Coquillettidia) fuscopennatus Theobald and 31 T. (Mansonioides) africanus Theo. After sorting by Dr. A. J. Haddow and chilling, the T. fuscopennatus were ground up in 5.0 ml. and the T. africanus in 2.0 ml. of physiological saline solution and the suspensions were then centrifuged. Groups of 6 mice were inoculated intracerebrally with Seitz-filtered and unfiltered suspensions. Nothing occurred in mice as a result of the inoculations with the suspension of T. africanus.

In the group of mice inoculated with the unfiltered suspension of T. fuscopennatus, 2 mice became paralysed on the 5th day and were sacrificed for subinoculation purposes and pathological studies. On the 6th day there were 3 additional paralysed mice, 2 of which died on the 9th day; on the 21st day there was 1 moribund mouse and 1 survivor in the group. In the 2nd passage in mice subinoculated intracerebrally with the Seitz-filtered supernate of a 10 per cent mouse-brain suspension, there was 100 per cent mortality and the average survival time was 6.8 days. The average survival times of mice in the next 3 intracerebral transfers of Seitz-filtered suspensions were 7.6, 5, and 6.2 days. Subsequently, transfers were made with filtered suspensions injected intraperitoneally in 0.06 ml.

quantities. The average survival time in the next 5 transfers was 4.0 days; from the 18th to 29th 2.2 days, and thereafter up to the 34th passage it was 2.0 days.

Isolation from the serum of a Mongoose.

On September 2, 1946, a mongoose (Ichneumia sp.) was trapped on the compound of the Institute and before being destroyed it was bled routinely for a yellow fever protection test. Fourteen-day old mice were used in the test (29), and on the 2nd day it was found that all the mice inoculated with the mongoose serum-yellow fever virus mixture were dead. With non-protective sera the incubation period in this test is at least 3 days, and deaths on the 2nd day are regarded as non-specific so far as yellow fever virus is concerned. In order to discover the reason for the "toxicity" of this serum, groups of adult mice were inoculated intracerebrally with a portion of the mongoose serum which had been kept in the refrigerator. A strain of virus was isolated directly from the serum and successful serial intracerebral transfers were made.

As an interesting sideline to this isolation of virus directly from the serum of the mongoose the following experiment was done. The brains and livers of the baby mice which had died on the 2nd day in the yellow fever protection text were ground up with yellow fever immune serum, centrifuged and incubated at 37°C. for 2 hours; groups of mice were then inoculated. Six adult mice inoculated intracerebrally with the suspensions of brains of the 14 day old mice were all dead on the 2nd day. ME virus was identified as the infectious agent and successful intracerebral transfers were made. From this experiment it may be seen that in animals infected with 2 viruses (in this case yellow fever and ME virus) 1 virus may be isolated by the neutralisation of the other by a specific immune serum.

Isolation from a human case of encephalitis.

The clinical syndrome of the illness is discussed in detail on p. 54. Isolations of virus were made in mice and a rhesus monkey. (This isolation was initiated by Dr. K. C. Smithburn and continued by the writer.)

In mice. Blood was taken from the patient on the 1st day of illness, and the serum was inoculated intracerebrally in 0.03 ml. quantities into each of 8 six-day-old mice and intraperitoneally in 0.1 ml. quantities into 8 other mice of the same age. Of the mice inoculated intracerebrally 1 died on the 1st day. On the 2nd day 2 of the mice died and 1 was incoordinated and had paralysis of the hind legs; 1 mouse died on the 4th day; 2 mice were sick on the 8th and 9th days and were sacrificed, and successful intracerebral transfers were made. There was 1 survivor on the 13th day.

Of the 8 mice inoculated intraperitoneally with the patient's serum 1 died on the 2nd day and was used for subinoculation purposes. The other 7 mice remained well for 30 days. From the dead mouse a successful intracerebral transfer was made, a 10 per cent Seitz-filtered suspension of the mouse-brain being used. By the 2nd passage there was 100 per cent mortality of inoculated mice.

The average survival time from the 2nd up to the 10th passage in mice inoculated with 10 per cent Seitz-filtered mouse-brain suspensions was 1.2, 1.4, 1.0, 1.4, 1.2, 1.0, 1.4, 1.0, and 1.0 days. From the 10th to the 26th passage (when mouse-

brain passage was stopped) the average survival time was usually less than 1 day. The average survival time of mice inoculated with the human strain of the virus was at first shorter than that of mice inoculated with any of the other strains except the mongoose strain. Apart from this the clinical signs in mice inoculated with mouse-brain suspensions of the human strain were similar in all respects to those produced by other strains of the virus.

In a rhesus monkey. On the 2nd day of illness the patient was again bled, and rhesus monkey 739 was inoculated intracerebrally with 0.1 ml. of the serum. The clinical disease in this and other inoculated rhesus monkeys is discussed on p.46 . Rhesus 739 was bled on the 8th day, and mice and rhesus monkey 600 were subinoculated with its serum. Mice inoculated intracerebrally with 0.03 ml. quantities of this serum behaved similarly to those receiving other strains of ME virus. Passages of 10 per cent Seitz-filtered mouse-brain suspensions were successful. The average survival time of the 1st groups of mice inoculated with serum of rhesus 739 was 1 day; in the next 6 passages, in which mouse-brain suspensions were used, the average survival time was less than 1 day. From the 8th to the 21st passage mice were inoculated intraperitoneally with mouse-brain suspension, and the average survival time remained at about 1 day. Mouse-brain passages were then discontinued, and mouse-brain virus was preserved by freezing and drying, and in 50 per cent glycerine.

Isolation from a rhesus monkey with fever but no paralysis.

On May 3, 1947, a rhesus monkey (823) , brought from the outdoor run to the laboratory, was bled for a yellow fever protection test and inoculated with a suspension of mosquitoes. This animal had been bled in March 1947 for a yellow fever protection test but had not been submitted to any other experimental procedures. On arrival in the laboratory on May 3 it had a temperature of 104°F. but little attention was paid to this as monkeys often show an elevation of temperature after the chase necessary to capture them in the outdoor runs. However, its temperature was 104.6°F. on the following day, and in view of this fact, Dr. K. C. Smithburn inoculated 6 mice intracerebrally with its serum obtained on May 3, which had been kept in a refrigerator overnight. One of the inoculated mice died within the first 24 hours, probably from trauma. Of the 5 remaining, 1 appeared sick on the 3rd day and exhibited convulsive movements, and 1 was paralysed on the 4th day. These mice were sacrificed for subinoculation purposes. Another mouse became sick on the 6th day and was sacrificed for pathological studies. Of the remaining 2 mice, 1 became paralysed on the 8th day and was still paralysed on the 21st day, at which time there was 1 well survivor of the group. There was 100 per cent mortality among the mice subinoculated intracerebrally with suspensions of the brains of sick and paralysed mice. The average survival time by the 3rd intracerebral passage of virus isolated from rhesus 823 was less than 24 hours (when a Seitz-filtered 10

per cent mouse-brain suspension was used for the subinoculations).

This isolation of virus from a second rhesus monkey is in striking contrast with the original isolation from the paralysed rhesus UR 22. In the latter instance no virus was isolated from the blood, but virus was recovered from both the cerebrospinal fluid and the spinal cord, whereas in the second instance virus was recovered from the blood. However, the first monkey was past the febrile stage of its illness (if, in fact, it had had fever) when the materials were taken for inoculation. Rhesus 823 was in the febrile stage of its illness when the serum from which virus was isolated was obtained. Rhesus 823 did not develop any paralysis but developed antibody to ME virus during convalescence(p.19).

IDENTITY OF THE STRAINS OF VIRUS

Within one year 6 strains of virus were isolated. Since (as will be seen later), (a) the human case and the pyrexial rhesus monkey from which strains of virus were isolated developed antibodies to ME virus during convalescence (p.19), and (b) unused monkeys have developed antibody to this virus while caged in the compound at Entebbe (p.60), there is sufficient evidence that these strains of virus did not originate in inoculated mice.

The identity of the 6 strains of virus was shown by cross neutralization tests. The techniques employed in these and other serological tests were based on methods used by Olitsky

and Harford (30) in virus neutralization tests with equine encephalitis viruses.

The virus neutralization test is used to demonstrate specificity of antibody for a known virus. Briefly, serum which is suspected of containing antibody is added to a suspension of known virus, or vice versa, suspensions of unknown viruses are added to sera containing known antibody. The serum-virus mixtures are usually allowed to stand at room or incubator temperatures for a certain time and animals (for the encephalitis viruses usually Swiss white mice) are then inoculated with these mixtures by a route which depends on the pathogenicity of the virus under test. Normal and immune control serum-virus mixtures are also set up and inoculated into groups of mice, the former to determine the titre of the virus suspension used and the latter to check the specificity of the reaction. The number of virus particles neutralized by the known and unknown sera may then by calculated from the mortality ratios of the inoculated mice (31), and the neutralizing effect of the unknown serum may be compared with that of the normal and immune control sera. Similarly the degree of neutralization of an unknown virus by a specific immune serum may be calculated.

In preliminary experiments the preinoculation and convalescent sera of inoculated monkeys were tested for their neutralizing effect on ME virus. The virus material for these tests was prepared by making a 10 per cent suspension of brains of infected mice in distilled water[1] and centrifuging it at low speed for 15 to 20 minutes. Ten-fold dilutions of the supernate were made, using as diluent 10 per cent serum-saline mixtures of each serum to be tested. To 0.5 ml. portions of each undiluted serum were added 0.25 ml. portions of the virus dilutions which had been made with the respective serum diluent. The mixtures were shaken, incubated for 1 hour and then inoculated

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1. Distilled water was used as diluent because we could not at this time be certain that any of our normal monkeys did not have antibody to ME virus in their sera.

intraperitoneally in 0.06 ml. quantities into mice in groups of 6.

In these initial experiments it was shown that when serial dilutions of ME virus (UR 22 monkey strain) were mixed with preinoculation and other normal non-immune monkey sera, titres averaging $10^{-7.2}$ were obtained. Serum taken from rhesus 692 30 days after subinoculation with material from UR 22 (the original paralysed monkey) neutralized at least 31,700 LD_{50} of ME virus, while serum taken from a monkey (621) 8 days after inoculation with mouse-brain ME virus neutralized 280 LD_{50} of virus. It appeared, therefore, from these preliminary experiments that an intraperitoneal neutralization test was satisfactory for studies to establish the relationship of the 6 agents which had been isolated.

By experiments using the technique described above, it was shown that convalescent serum from a monkey (692) which had been inoculated with UR 22 monkey virus neutralized at least 10,000 LD_{50} of the viruses isolated from the T. fuscopennatus, the Taeniorhynchus spp. and the mongoose. The final proof of the identity of these 3 agents with the UR 22 monkey virus was established by the following experiment.

Sera from 3 rabbits (169, 170 and 171) were each tested for neutralizing antibody against the viruses isolated from rhesus UR 22, T. fuscopennatus, and the Taeniorhynchus spp. The titres obtained demonstrated that none of the rabbits had neutralizing antibodies to any of these viruses. Rabbit 169 was then hyperimmunised against the UR 22 virus, rabbit 170 against the T. fuscopennatus virus and rabbit 171 against the virus isolated from the mixed batch of Taeniorhynchus spp. This was accomplished by inoculating the rabbits at 3-day intervals with 1 ml., 1 ml., and 0.75 ml. of 10 per cent mouse-brain suspensions of one or other of the 3 agents. Ten days after the last injections of virus the rabbits were bled and using the intraperitoneal test already described a cross neutralization test was made. In the results of this experiment as presented in Table 1, the LD_{50} of virus neutralized in each case is calculated from the titre of each virus mixed with the homologous normal serum only. The titres of the heterologous normal sera were of the same order as those pre-

sented.

(TABLE 1.)

The identity of the agent isolated from the mongoose and the UR 22 monkey virus was established by a second similar cross neutralization test. Rabbit 168 was hyper-immunised by repeated subcutaneous injections of mouse-brain passage virus which originally had been isolated from the mongoose serum. Virus-serum mixtures were prepared as described above and groups of mice were thereafter inoculated with the mixtures. It was shown that the convalescent serum of rabbit 168 neutralized more than 1,780 LD_{50} of both the UR 22 monkey and mongoose viruses, and that UR 22 monkey virus immune serum neutralized more than 31,700 LD_{50} of the mongoose virus.

A similar type of experiment was done with the rhesus 823 strain of virus. The rhesus 823 mouse-brain virus was completely neutralized by the UR 22 monkey strain immune serum, but not by normal monkey sera. Inactivated acute-phase[1] and convalescent sera of rhesus 823 were tested against this virus and against the UR 22 monkey strain of ME virus. The acute-phase serum had no protective power against either the rhesus 823 or UR 22 monkey strains of ME virus, while the convalescent serum of rhesus 823 neutralized more than 10,000 LD_{50} of both. Moreover, the rhesus 823 monkey virus was again completely neutralized by UR 22 monkey strain immune serum. This virus was therefore proved to be another strain of the ME virus.

The serological identity of a virus isolated from a human case of encephalitis with that of other strains of ME virus was accomplished as follows. A 1 in 100 suspension in saline solution was made with the brain of a sick mouse which had been inoculated with 5th passage mouse-brain suspension of the infective agent recovered directly from the human case. The mouse-brain virus suspension in 0.25 ml. quantities was added to 0.5 ml. portions of known normal and immune serum. The virus-serum mixtures were allowed to stand at room temperature for 20 mins. and mice in groups of 6 were each inoculated intraperitoneally with 0.06 ml. of each mixture. All mice remained well after inoculation with the mouse-brain virus suspension mixed with convalescent serum from a monkey (692) which had been inoculated with the UR 22 monkey strain of ME virus. There was no neutralization of the human strain of virus by sera from known non-immune animals.

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1. At the time when this experiment was made the marked stability of ME virus was not known. It is conceivable that inactivated acute-phase serum from rhesus 823 contained active virus.

A similar experiment was done with 2nd passage mouse-brain virus recovered from the human patient through rhesus monkey 739. The virus was again neutralized by serum from a monkey (692) known to be immune to the UR 22 monkey virus, but not by normal sera.

The final proof of the identity of the infective agent isolated from the human was achieved when it was shown that the patient's convalescent serum neutralized both the virus isolated from his own serum and from the UR 22 monkey. Inactivated 2nd day acute-phase serum from the patient had no such neutralizing power. This latter serum was inactivated by heating at 64°C. for 1 hour. At the time of this test the pronounced stability of ME virus was not known, and it is conceivable that active virus may have been present in the "inactivated" specimen. Accordingly about a year after these tests were made, another protection test was done, in which the neutralization effect of human immune serum on the UR 22 strain of virus was compared with that of (a) an immune monkey serum, (b) a normal monkey serum, and (c) serum taken from the human on the 3rd day of illness and stored in the refrigerator. No virus was isolated from this 3rd day acute-phase serum, and any objection such as might be raised in the case of the original protection tests could not apply. The results of these intraperitoneal protection tests are presented in Table 2, which shows that the convalescent human serum protected mice against a test dose of 25,000 LD_{50} of UR 22 strain of ME virus and against at least 3,170 LD_{50} of the strain isolated from the human patient.

(TABLE 2.)

From the above cross-neutralization experiments it may be seen that the agents isolated from (a) the human case of encephalitis, (b) the paralysed rhesus monkey (UR 22), (c) the febrile rhesus monkey (823), (d) the mongoose, (e) from the T. fuscopennatus and (f) the mixed group of Taeniorhynchus mosquitoes are all serologically indistinguishable. They may be considered as strains of the virus of Mengo encephalomyelitis.

The UR 22 monkey strain of virus has been used for all the studies reported in this thesis unless otherwise stated.

RELATIONSHIP OF ME VIRUS TO OTHER NEUROTROPIC VIRUSES.

Viral encephalitides immune sera

Experiments using the technique described above were done with the UR 22 monkey strain of virus and anti-sera to various viruses isolated in Uganda. (Table 3) From the results of these experiments it may be seen that the titres of ME virus, when it is mixed with sera immune to yellow fever, Semliki Forest, Bunyamwera, Bwamba Fever or Uganda S. viruses are of the same order as titrations of this virus in normal control sera.

(TABLE 3.)

Studies were made to find out if ME virus was identical with certain other human neurotropic viruses. In these experiments brains of mice paralysed as a result of inoculation with ME virus were suspended in 10 per cent normal monkey serum in physiological saline solution. Serial decimal dilutions of the supernates were made and to 0.05 ml. of each serum under test, 0.25 ml. of one or other of the dilutions of the virus was added as indicated in Table 4. The virus-serum mixtures were shaken and allowed to stand at room temperature for 40 minutes prior to intraperitoneal inoculation of mice. Each mixture was tested in a group of 6 mice; the inoculum was 0.06 ml. for each mouse. In all cases end-point titrations of the immune-serum-virus mixtures were not obtained. The anti-sera to Eastern equine, Western equine, St. Louis, West Nile, and Japanese B. Encephalitis were prepared in 1945 by repeated subcutaneous inoculations of the respective virus

into rhesus monkeys. These sera were stored in the dry state and rehydrated before use. Tests were also made with hyper-immune sera to the following: louping ill (supplied by Dr. D. G.ff. Edward), lymphocytic choriomeningitis (supplied by Dr. F. O. MacCallum), Ilheús and Venezuelan encephalitis (supplied by Dr. T. P. Hughes) and Far Eastern (Russian) encephalitis and Sabin's B virus (prepared in rabbits with virus supplied by Dr. P. K. Olitsky), the results of these tests are presented in Table 4.

(TABLE 4.)

From all these experiments it was shown that while sera of rhesus monkeys which were immune either as a result of natural infection (722) or inoculation with ME virus, could neutralize 317,000 or more LD_{50} of ME virus, sera from animals specifically hyperimmunised against the viruses listed in Table 4 had no such neutralizing property.

It may be concluded, therefore, that ME virus is not identical with any of the above human encephalitic viruses.

Poliomyelitis immune sera.

Tests were also undertaken to test the neutralization capacity of serum containing antibodies to one or other of the types of poliomyelitis virus. Experimental methods similar to those described above were employed (except that 0.2 ml. of each virus dilution was added to 0.4 ml. quantities of serum), and tests were made with convalescent sera from monkeys which had been immunised against one or other of the following

strains of poliomyelitis virus: the Lansing (18), Yale SK (19), and MEF-1 (32) rodent adapted types, the Brunhilde (20), and the New York City-1944 monkey adapted types[1]. The results of this test are presented in Table 5.

(TABLE 5.)

It may be seen that none of the poliomyelitis immune sera neutralized ME virus. Similar results were obtained when pools of poliomyelitis convalescent human sera were tested.

Mouse encephalomyelitis immune sera.

Specific immune sera to the TO, FA and GD VII[2] strains of mouse encephalomyelitis virus were also tested for any neutralizing effect on ME virus.

(TABLE 6.)

From the results of these tests, which are presented in Table 6, it may be seen that ME virus is not serologically related to any of the strains of the encephalomyelitides of mice.

OTHER AGENTS

No von Economo's encephalitis immune serum nor anti-sera to Swineherd's disease (24) were available for test, but the

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1. The Lansing immune serum was supplied by Dr. I. M. Morgan; the Yale-SK and New York City-1944 strain hyperimmune sera were supplied by Dr. J. L. Melnick. Dr. P. K. Olitsky provided convalescent serum from a monkey inoculated with the MEF-1 strain of poliomyelitis. Dr. C. W. Jungeblut supplied the Brunhilde immune sera.
2. The TO (Theiler's original) strain (33) immune serum was supplied by Dr. P. K. Olitsky, the GD VII strain (34) immune serum by Dr. J. Warren and immune serum to the FA strain (34) by Dr. C. W. Jungeblut.

host range and pathogenicity of ME virus were of a nature to make an adequate differentiation from these and other viruses against the immune sera of which tests were not made.

SUMMARY OF PRELIMINARY SEROLOGICAL STUDIES

From the above serological studies it was concluded that ME virus was not identical with any of the agents against the anti-serum of which it was tested. From studies of its pathogenicity it appeared to differ from all other known viruses[1] and it was therefore considered that this agent which in 1946 was called Mengo encephalomyelitis virus was hitherto unknown.

RELATIONSHIP OF ME VIRUS TO EMC, COL SK AND MM VIRUSES

At the Fourth International Congress on Tropical Medicine and Malaria (Washington 1948) some of the studies on ME virus were presented (35). In the discussion which followed this presentation certain similarities between ME virus and the recently isolated virus of encephalomyocarditis (EMC) were suggested. Accordingly, some convalescent serum from a human case of ME virus infection was given to Dr. J. Warren (of the Army Medical Center, Washington, D. C.) who showed by neutralization tests that that serum neutralized more than 1,000 LD_{50} of EMC virus (36).

It had been reported by Warren and Smadel (37) that EMC virus exhibited cross-neutralization with the Columbia SK and

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1. The report of the isolation of the virus of encephalomyocarditis (10) had not come to hand.

MM murine strains of poliomyelitis virus. From the experiments which had been made with ME virus it was not possible to demonstrate any neutralization of ME virus with various poliomyelitis anti-sera. In view of the report by Warren on the neutralization of EMC virus by ME immune sera, and of the relationship of EMC virus to so called murine strains of poliomyelitis it became necessary to plan a complete study of the relationship of ME, EMC, Col SK and MM viruses and other rodent adapted strains of poliomyelitis virus.

Before discussing the serological relationship of this group, a brief resume of the isolation of the SK, MM and EMC viruses will be given.

Columbia SK and Yale SK viruses

The SK strain of poliomyelitis virus was originally isolated in monkeys from the faeces of an abortive case of poliomyelitis (38).

Columbia SK virus. Jungeblut and Sanders (11) in 1941 claimed that they had transmitted the SK strain from monkeys to mice by the intermediary passage through cotton-rats. They stated that the symptoms and lesions produced by the murine agents compared in all respects with poliomyelitis in man.

This murine virus which they isolated differed, however, characteristically from the SK virus with which their experiments were initiated, and was also unlike the Lansing type of rodent adapted poliomyelitis virus. Jungeblut et al (11) were of the opinion that the agent they were dealing with was

a high titre viriant of the parent SK virus.

Yale SK virus. Later, in the New Haven Laboratory, the SK virus was adapted to cotton-rats and mice (19). Following this adaption the Y-SK virus (as it is now called) behaved like the Lansing type of poliomyelitis virus with regard to pathogenicity, host range and pathological manifestation and was antigenically related to the original SK virus isolated in monkeys.

MM virus.

In 1943, Jungeblut and Dalldorf (12) isolated a virus from the brain of a hamster which died 19 days after inoculation with material from the brain-stem and cord of a fatal case (MM) of poliomyelitis. At the same time they isolated a strain of this virus from the brain of a house mouse found dead in the home of the fatal case. Many contradictory and irregular results have been published on the immunological relationships of these 2 agents. On original isolation they were said to be completely inactivated by anti-sera to Theiler's virus of mouse encephalomyelitis (12), however, Dalldorf and Whitney (39) however using cross neutralization and cross immunity tests, showed that there was no relationship between MM virus and Theiler's original TO strain, but by compliment fixation claimed that a relationship existed between the GD VII strain of Theiler's virus and the MM virus. Schatz and Plager (40) quoting Dalldorf's unpublished studies recently stated that MM virus is serologically similar to Col SK virus.

Although the original material from MM contained an agent which produced paralysis in a rhesus monkey, the MM virus derived from the hamster brain has apparently not been tested to determine whether it was related to the monkey pathogenic material in the original human tissue.

The virus of encephalomyocarditis.

In December 1944 a filter passing agent was isolated by Helwig and Schmidt (10) from the spleen and pleural fluid of a chimpanzee which died suddenly at the Anthropoid Ape Research Foundation, Dania, Florida. This animal was said to have had no history of illness. Autopsy showed bilateral hydrothorax, pulmonary oedema, pericardial effusion and an intense diffuse interstitial myocarditis. The brain was said to be negative and the spinal cord was not examined. A virus was isolated in mice, and by the 2nd passage of a mixture of viscera, all inoculated mice were paralysed by the 4th day and dead by the 8th day. Passage was maintained by various routes through a series of 122 mice and regularly produced paralysis followed by death, or by apparent recovery after a week or two. At necropsy, interstitial myocarditis was found in almost all animals.

Warren and Smadel (41) found this virus unrelated immunologically to a number of other viruses and designated it the virus of encephalomyocarditis in virtue of the fact that both encephalitic and myocardial lesions were produced by inoculation of this agent into experimental animals.

SEROLOGICAL IDENTITY OF ME, EMC, COL SK AND MM VIRUSES.

Cross neutralization tests with ME, EMC, Col SK, and MM viruses.

Viruses. The UR 22 monkey strain of virus was employed in these experiments. The 19th mouse passage of this virus, lyophilised, was brought from Uganda, Africa to the I. H. D. Laboratory of the Rockefeller Foundation, New York. EMC virus was supplied by Dr. J. Warren as lyophilised mouse-brain virus, 54th passage. Dr. C. W. Jungeblut provided glycerolated mouse-brains infected with Col SK virus (396th passage) or with MM virus (167th passage). All these viruses were passed intraperitoneally in mice 3 or more times prior to being employed for the immunisation of monkeys. For the cross-neutralization tests ME virus was used in the 26th mouse passage, EMC virus in the 66th, Col SK virus in the 401st and MM virus in the 171st passage.

Monkeys and mice inoculated with these viruses were kept in strict isolation although no evidence exists which indicates that there is cross infection with this group, except possibly through cannibalism amoung the infected mice. Separate isolation rooms were used for mice inoculated with ME and EMC viruses, and mice inoculated with Col SK and MM viruses were kept in isolation cubicles. Monkeys were separately isolated in cubicles during and after immunisation.

Sera. Rhesus monkeys were employed for the production of immune sera to the viruses under test. Prior to inoculation approximately 50 ml. of blood were withdrawn from each of the monkeys. In all instances the monkeys were immunised by the inoculation of the supernate of lightly centrifuged 10 per cent mouse-brain virus suspensions, Rhesus 4811 was inoculated subcutaneously with 0.25 ml. of mouse-brain suspension of ME virus (24th passage), and 10 days later it was given a second inoculation of 5 ml. of the same suspension intraperitoneally. Rhesus 4781, 4789 and 4795 were immunised against EMC, Col SK and MM viruses respectively by initial subcutaneous inoculations of 0.25 ml. of a 10 per cent mouse-brain suspension of the respective virus and subsequent intraperitoneal inoculations at 8- and 10-day intervals of 2.0 ml. and 5.0 ml. of the same suspensions of virus. The suspensions (EMC virus, 64th passage; Col SK, 400th passage; and MM, 171st passage) were stored in lusteroid tubes at -70°C. during the intervals between the immunising inoculations, The monkeys were bled 7 days after the last inoculation and the antisera were stored in a refrigerator at 4°C.

Test Procedure. An intraperitoneal neutralization test similar to that previously employed in studies of ME virus was used. The brains, however, were suspended in 0.2 per cent bovine plasma albumin in buffered saline solution[1]. The

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1. In all this series of experiments o.2 per cent bovine plasma albumin (Armour) in saline solution buffered at pH 7.4 to 7.6 was employed as the diluent (42).

suspensions were centrifuged at approximately 1,500 r.p.m. for 20 minutes. Serial decimal dilutions of the supernates were made with the bovine albumin diluent. To 0.5 ml. of each serum under test, 0.25 ml. of one or other of the dilutions of virus was added. The virus-serum mixtures were shaken and allowed to stand at room temperature for 40 minutes, prior to intraperitoneal inoculation of mice. Each mixture was tested in a group of 6 mice[1]; the inoculum was 0.06 ml. for each mouse.

The results of this cross-neutralization experiment with ME, EMC, Col SK and MM viruses are presented in Table 7, In each case, each virus was tested against the preimmunisation and convalescent homologous and heterologous sera of the rhesus monkeys.

(TABLE 7.)

It may be seen from Table 7 that there is complete cross-neutralization between ME, EMC, Col SK and MM viruses. The observation of Warren and Smadel (41) that EMC virus is related to the Col SK and MM agents is also confirmed.

RELATIONSHIP OF ME AND COL SK VIRUSES

and

THE YALE-SK AND LANSING STRAINS OF POLIOMYELITIS VIRUS

It has been shown that ME virus is not neutralized by antisera of various strains of poliomyelitis virus including the Y-SK strain (Table 5). Furthermore, from other experiments presented above (Table 7) a close relationship was demon-

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1. Swiss white mice of the Bar Harbor, Maine, stock were employed in this series of experiments; in the subsequent experiments Swiss white mice of the Rockefeller Institute or of the Bar Harbor stocks were used. In any one experiment 3- to 4-week-old mice of the same stock were employed.

strated between ME virus and the Col SK virus. Since it had been claimed that Col SK was immunologically related to the SK strain of poliomyelitis virus (43), it seemed essential to study further the relationship of Col SK and ME virus and the Y-SK and Lansing adapted strains of poliomyelitis.

Neutralization tests with ME virus. The following sera were tested for their neutralizing capacity on ME virus: (a) sera from monkeys immunised against 2 Columbia laboratory strains of the SK and Aycock (11) viruses; (b) sera from a rabbit immunised against MM virus;[1] (c) convalescent sera from monkeys which had been immunised against one or other of the following strains of poliomyelitis virus - the Lansing, Yale-SK from Yale laboratory and MEF-1 (32) rodent-adapted strains, the Brunhilde (20) and the New York City-1944 monkey-adapted strains. The results of this test are presented in Table 8.

(TABLE 8.)

It may be seen that immune sera to the Yale laboratory SK, the Lansing and the MEF-1 rodent-adapted strains of poliomyelitis virus and to the Brunhilde and New York City-1944 monkey-adapted strains of poliomyelitis virus do not neutralize ME virus. ME virus is neutralized in high titre, by sera containing antibodies to the Columbia strains of SK virus. Since SK virus immune serum from Yale laboratory does not neutralize ME virus, and SK virus immune serum from the Columbia laboratory neutralized ME virus in high titre, it must be concluded that the Columbia-

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1. Kindly supplied by Dr. C. W. Jungeblut.

SK virus is a different virus from the original Yale-SK poliomyelitis virus with respect to this serological reaction. ME virus was also neutralized by the antiserum for the Columbia strain of Aycock virus[1]. It had already been shown by Jungeblut and Sanders (11), that the Columbia strain of SK virus was related to the Columbia strain of Aycock virus.

Neutralization test with Col SK virus. To confirm the observation that Yale-SK and Columbia-SK immune sera are serologically distinct, these sera were tested against Col SK virus. No units of Col SK virus were neutralized by (a) a pool of sera from convalescent monkeys which had been hyperimmunised with the Yale-SK and the Lansing strains of poliomyelitis virus[2], or (b) antisera (Rh. 3758) to the New York City-1944 strain of monkey-adapted poliomyelitis virus. But Col SK immune serum (Rh. 4789) neutralized 100,000 LD_{50} of Col SK virus.

Neutralization tests with the Lansing and Yale-SK rodent-adapted strains of poliomyelitis virus. Tests were undertaken to determine the neutralizing capacity of ME and Col SK virus immune sera for the Lansing and Yale-SK viruses; the latter was received from the Yale laboratory. Since all rodent-adapted strains of poliomyelitis virus are serologically related to the Lansing type (20) a study of only 2 strains was undertaken. Furthermore, as Warren and Smadel (41) had already

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1. Dr. W. L. Aycock was unable to supply any convalescent serum to the Aycock strain from his laboratory for confirmation of this result.
2. Supplied by Dr. M. Theiler.

shown that EMC virus was unrelated to the Yale-SK and Lansing strains of poliomyelitis virus, and since EMC, MM, Col SK and ME viruses are serologically similar, antisera to only the Col SK and ME viruses were tested to study the relationship of the group containing ME, EMC, Col SK and MM viruses to rodent-adapted strains of poliomyelitis virus.

Viruses. The Yale-SK virus (Yale-SK M9) was supplied as infected mouse cords in the frozen state by Dr. J. L. Melnick. A suspension of those cords was passed intracerebrally in mice, and the brains and cords of paralysed mice (after 1 mouse passage in the I. H. D. Laboratory) were employed in the neutralization test. The Lansing virus (A 2891 AV 2) was supplied by Dr. M. Theiler as a suspension of mouse-brains in the frozen state. This suspension was passed once intracerebrally in mice, and the brain and cord of a paralysed mouse were used in the neutralization test.

The method of carrying out the intracerebral neutralization tests for this study was essentially the same as that employed by other workers in this field. To 0.3 ml. of each serum was added 0.3 ml. of one or other of the decimal dilutions of virus suspensions as indicated in Table 9. Groups of 10 mice were inoculated intracerebrally with 0.03 ml. of each virus-serum mixture which had been allowed to stand at room temperature for 1 hour prior to inoculation. The inoculated mice were observed for 56 days.

(TABLE 9.)

From the foregoing table it may be seen that neither ME nor Col SK virus immune sera have any neutralizing effect on the Lansing or Yale-SK strains of poliomyelitis virus comparable to that of the homologous immune sera of the latter viruses. It may be concluded, furthermore, that neither Col SK virus nor ME virus is antigenically related to the rodent-adapted strains of poliomyelitis virus.

CONCLUSIONS FROM SEROLOGICAL TESTS

From the results of these tests it would appear that: (a) ME virus is serologically closely related to Col SK, MM and EMC viruses, (b) ME and Col SK viruses bear no antigenic relationship to the Lansing, Yale-SK (from Yale laboratory) or MEF-1 strains of the rodent-adapted type poliomyelitis, (c) ME virus is not neutralized by convalescent serum from monkeys immunised against the Brunhilde type or other monkey-adapted strains of poliomyelitis, (d) ME virus is not serologically related to any of the other encephalitis viruses against the immune sera of which it has been tested, (e) the Col SK and MM strains of virus are serologically identical. Since Col SK virus bears no antigenic relationship to poliomyelitis virus, it is reasonable to suggest that MM virus is also not serologically related to poliomyelitis virus. The evidence presented does not support the contention that Columbia-SK and MM viruses are rodent-adapted strains of poliomyelitis virus.

Evidence is also at hand, by challenge of vaccinated

animals, to show that ME, EMC, and Col SK viruses are also related immunologically.

PATHOGENIC PROPERTIES OF THE ME, EMC, COL SK AND MM GROUP

Since it has been demonstrated that the above group of agents are closely related antigenically it might be expected that their pathogenic properties would be very similar. The only members of this group which have been studied intensively are ME and Col SK viruses. The results of experiments with the former virus will be presented in detail and only brief mention will be made of studies on the other agents where they are confirmatory or at variance with the studies made with ME virus.

PATHOGENICITY FOR MICE

ME virus. The human, monkey, mongoose and mosquito strains of ME virus were first demonstrated by virtue of their pathogenicity for mice. Apart from some slight variations in the incubation periods and average survival times of mice inoculated with early-passage virus, the behavior of all 6 strains of ME virus in mice has been identical. All the strains caused 100 per cent mortality in inoculated mice by the 2nd intracebral passage, except the one isolated from the mixed group of Taeniorhynchus mosquitoes. In the latter case 100 per cent mortality occurred by 3rd and 4th transfers in 2 lines of passage and by the 6th transfer in the line with which serial passages were continued. In Table 10 is presented a comparison of the average times of survival in the early passages

in which mice were inoculated intracerebrally.

(TABLE 10.)

In the early passages in adult mice, the first clinical evidence of illness was usually paralysis of one or more limbs. As a rule both hind limbs exhibited a flaccid paralysis. If the mouse survived the paralysis of the hind limbs for more than 4 days, the front limbs usually then became paralysed. In some of the early passages, and with small quantities of virus in later passages, mice occasionally remained paralysed throughout the period of observation of 21 to 30 days. In general, however, after the 2nd mouse passage, prostration and death supervened rapidly after the onset of symptoms. Following intracerebral inoculation of relatively large doses of early-passage virus, the symptoms first appeared 48 to 56 hours after inoculation and all mice were dead with 72 hours. Paralysis in these mice was sometimes, but not always, preceded by a rough appearance of the fur. With small doses of virus (about 100 LD_{50}), sufficient to cause death of all mice, the incubation period was usually prolonged to 5, 6, or 7 days, and death, which usually occurred within 2 to 3 days after the onset of symptoms, was sometimes delayed until the 10th to the 16th day. Mice which have been paralysed for a few days were incontinent. The tail appeared to escape paralysis. With later-passage virus, death usually occured within 24 to 48 hours after inoculation and without any preceding evidence of paralysis or sickness.

Intraperitoneal inoculations produced a similar sequence

of events. When early-passage virus was used, the incubation period was about 3 days if relatively large doses of virus were given, and between 5 and 14 days if high dilutions of virus were employed. With later-passage virus mice became paralysed and died within 48 hours. By intranasal or subcutaneous inoculation of virus the incubation period was prolonged by 24 to 48 hours over that following intraperitoneal inoculation. Mice inoculated subcutaneously became paralysed and died a little earlier than mice inoculated intranasally.

The efficiency of various routes of inoculation was tested with both early- and late-passage virus. For this purpose 10 per cent suspensions of infective mouse-brain tissue were prepared and centrifuged at low speed for 20 minutes. Serial ten-fold dilutions of the supernates were made, using 10 per cent normal serum-saline solution[1] as diluent. The results of these experiments are presented in Table 11.

(TABLE 11.)

Mice 4 to 6 days old died within 24 to 48 hours after intraperitoneal inoculation with 0.06 ml. quantities of 10 per cent mouse-brain passage virus. There was no significant difference in the susceptibility of mice 3 to 12 weeks of age to the extraneural inoculation of this virus, but the average survival time in old mice inoculated with 10 per cent mouse-

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1. The term 10 per cent serum-saline indicates a mixture of 10 volumes of whole normal monkey serum and 90 volumes of 0.86 per cent sodium chloride solution in distilled water.

brain virus was twice as long as that of 21-day-old mice inoculated with the same suspension.

EMC, Col SK and MM viruses. The behaviour of these agents in mice is practically identical with that of ME virus. EMC (44) and Col SK (11) viruses yield titres of 10^{-7} or more and are highly pathogenic by intracerebral, intranasal, intraperitoneal and other peripheral routes. MM virus reaches intracerebral titres of 1×10^{-11} (for the 0.03 ml. dose) and intraperitoneal titres of 1×10^{-9} (12).

Distribution of virus in tissues of inoculated mice.

Studies were made to determine the distribution and relative concentration of ME virus in the blood stream and viscera of inoculated mice. One mouse of the 15th passage was bled from the heart 48 hours after intraperitoneal inoculation and before the appearance of any signs of illness. A group of mice was immediately inoculated intraperitoneally with the blood of this animal, each mouse receiving 0.06 ml. The remainder of the specimen was used to make serial dilutions of the blood, with normal saline solution as diluent. The titre of the virus in the blood stream was $10^{-4.1}$, showing that this virus is present in high concentration in the blood stream of inoculated mice before the onset of objective signs.

In another experiment 2 paralysed mice of the 34th passage were sacrificed. Their brains, spinal cords, spleen, kidneys, livers, hearts, and lungs were removed aseptically, using clean instruments for each tissue from each mouse. The

entire organs, (with the exception of the liver, from which portions of all lobes were taken) were weighed to the nearest milligram and suspended in 10 per cent serum-saline solution to make 10 per cent suspensions. The preparations were centrifuged simultaneously at low speed for 10 minutes, and the supernates were passed through Seitz E.K. pads. Groups of 6 mice were inoculated intracerebrally with serial decimal dilutions of each tissue suspension. The results of this experiment are presented in Table 12.

(TABLE 12.)

The high concentration of virus in the brain and cord, as compared with other organs, shows how strictly neurotropic this virus is. The presence of virus in relatively low concentrations in organs other than the central nervous system could be accounted for by the virus content of the blood.

Col SK virus. Jungeblut and Sanders (11) reported recovery of Col SK virus from the brain, cord, spleen, liver, suprarenal and heart's blood of mice sacrificed at the height of paralysis. They do not, however, present any data regarding the titre of virus in these tissues.

Multiplication of virus in the brains of mice.

The rate of multiplication of the virus in the brains of mice and the quantity of virus circulating in the blood after intranasal inoculation were studied. In the first experiment 18 mice were each inoculated intranasally with 0.03 ml. of the unfiltered supernate of a 10 per cent suspension of 93rd-

passage mouse-brain virus. After 30 minutes, 1, 3, 4, 8, 11, 24, 48 and 72 hours, 2 mice were anaesthetised and bled from the heart into syringes which had been rinsed out with heparin. The mice were then sacrificed and their brains were removed. The blood specimens and 10 per cent suspensions of the brains of each pair were separately pooled and centrifuged for 15 minutes at low speed. Groups of mice were inoculated with undiluted plasma and with various dilutions of the unfiltered supernates of the brain suspensions. Results of the experiments are shown in Table 13. (expt. No. 1)

It may be seen that virus was present in the circulation within half an hour of intranasal instillation of virus and could thereafter be demonstrated in the blood up to the 72nd hour. On the other hand, no virus could be demonstrated in the brains of mice until the 4th hour following inoculation, after which time rapid multiplication of virus occurred.

In the second experiment (Table 13) 42 mice were inoculated intranasally with a similar dose of virus, and 6 of these mice were sacrificed at 30 minutes, 2, 4, 6, 24, and 48 hours. At each time interval 6 mice were bled from the heart, the blood specimens were separately pooled and the sera tested in various dilutions in separate groups of mice. The mice of each group were exsanguinated after bleeding from the heart; the brains were removed, pooled, and suspended in saline solution to make 10 per cent suspensions and the supernates

were titrated in groups of mice. In this experiment virus was again demonstrated in the serum within 30 minutes after intranasal inoculation. The virus content of the blood stream rose rapidly to its maximum titre in about 4 to 6 hours. No virus was demonstrated in the brain until 6 hours after inoculation, but from then on multiplication of virus there took place rapidly. Further experiments are required before it can be determined whether the rising titre of virus in the blood is due to absorption of virus from the nasopharynx or elsewhere, prior to its localisation and multiplication in the central nervous system.

Infection by contact.

The possibility of infection by contact was investigated in several experiments. Six mice inoculated with ME virus were added to a box of marked normal mice. When the inoculated mice became paralysed, a transfer was made, and the six marked mice were caged with a group of freshly inoculated mice. The experiments were carried out with both male and female groups. Of a total of 18 normal female mice exposed to 204 infected female mice in three experiments, 2 became infected (18 per cent); and of 18 normal male mice exposed to 198 infected male mice, 13 became infected (72 per cent). This higher incidence of contact infections among males exposed to infected males suggests the transmission of virus might be caused by cannibalism, which was in greater evidence among male than among female groups. In another experiment

6 normal female mice were caged with 6 infected males and vice versa. In the box containing the female contacts, no infection took place; of the male contacts, 1 became infected. In all these experiments there was overcrowding of mice, since 12 mice were caged in boxes designed to house 6 mice only. When there is no overcrowding, contact infections do not occur. For example, in one experiment, in which mice of both sexes were used, 6 infected mice were added to a spacious box which contained 24 normal mice. No contact infections occurred, nor under these conditions was there any evidence of fighting or cannibalism. A final experiment was done to determine whether contact infections were likely to occur among mice in groups of 6 and thus possibly influence end point titres. Twelve groups of 6 mice each, were inoculated in the following way. In each of 2 groups, 1 mouse was inoculated and the other 5 were left as contacts; in the next 2 groups 2 mice were inoculated and 4 served as contact. In the case of the boxes with 3 inoculated mice and 3 contacts, 4 groups of mice were used. There were no contact infections in these groups; therefore it would appear that under the conditions in which experiments have been made contact infections do not take place.

Col SK and MM viruses. Although no experiments have been recorded of contact infections with any of the other agents of this group, it has been demonstrated that both Col SK (11, 45) and MM (12) viruses are invasive by feeding. This finding is in agreement with the suggestion that canibalism may have ac-

counted for some of the contact infections found to occur with ME virus.

PATHOGENICITY FOR GUINEA PIGS

ME virus. Guinea pigs are susceptible to the inoculation of ME virus by the intracerebral, subcutaneous, intraperitoneal and intradermal routes. Juvenile guinea pigs are more susceptible than older ones. Two young guinea pigs (142 and 143) were inoculated intracerebrally with 0.15 ml. of a pool of the unfiltered supernate of suspensions of brain and cord of the paralysed rhesus monkey (UR 22) from which ME virus was first isolated. Neither of these animals showed any objective signs as a result of the inoculation. They had fever on the 3rd to 5th and 4th to 7th days respectively, after inoculation. Circulating virus (identified as ME virus) was demonstrated in the serum of guinea pig 142 on the 3rd to 5th days. This animal died after cardiac puncture on the 9th day. Serum taken from the other guinea pig (143) on the 24th day after inoculation contained antibody which neutralized ME virus.

Two guinea pigs (173 and 174) were inoculated intracerebrally with 0.1 ml. of serum from rhesus 739, which, as a result of inoculation, was circulating the human strain of the virus. The 2 animals were dead within 24 hours and 48 hours, respectively. The virus was passed serially to 2 other guinea pigs (175 and 176) which were found dead within 24 hours. In all cases ME virus was identified in the brains of the guinea pigs. A 3rd intracerebral passage of this line of virus was made by the subinoculation of guinea pig 177, which died on the

3rd day without having exhibted any paralysis.

Of 5 guinea pigs inoculated intracerebrally, intraperitoneally or subcutaneously with mouse-brain passage ME virus, 1 which was inoculated subcutaneously died on the 8th day after inoculation. The others survived or were sacrificed for subinoculations. All of these animals exhibited fever for varying periods up to the 5th day after inoculation. Circulating virus was present for the first 3 days in the sera of all animals which survived, and virus identified as that of ME was reisolated from the brains of all sacrificed guinea pigs. In none of these animals was any paralysis seen.

In only 3 of the 22 inoculated guinea pigs was paralysis observed. Two of those which became paralysed were inoculated intracerebrally with 2nd guinea-pig-brain passage virus suspensions. Both developed paralysis of the hind limbs on the 2nd day after inoculation, one of them (148) died on the 2nd day. A suspension of the brain of the latter was inoculated intracerebrally into guinea pig 341. This animal developed fever and weakness of the hind quarters on the 4th day, and complete paralysis of the hind limbs set in on the 5th day. By the 8th day there was also paralysis of the left front limb. The animal was sacrificed on the 9th day for histological material.

<u>EMC virus.</u> Warren (45) has noted that guinea pigs developed a mild infection characterised by fever from the 1st to the 5th day after inoculation with EMC virus, and that only an occasional guinea pig became paralysed. All animals developed

infection, however, as evidenced by the appearance of neutralizing antibodies.

Col SK and MM virus. Early attempts to transmit the Col SK virus to guinea pigs were unsuccessful (11), but later a fixed strain of cavian passage virus was established (46-48) which, according to Jungeblut et al. differed in certain respects from their murine strain.

PATHOGENICITY FOR RABBITS

ME virus. The pathogenicity of ME virus has been studied in relatively few of these animals. For the purpose of studying the relationship of certain of the strains of this virus (p. 18) rabbits were inoculated subcutaneously at 3-day intervals with massive doses (over 33,300,000 mouse-intracerebral LD_{50}) of virus. None of these rabbits showed any clinical reactions, but all developed highly protective sera as the result of the inoculations.

Rabbits would appear to be insusceptible to the intracerebral inoculation of mouse-brain passage virus. While virus was recovered up to the 3rd day after inoculation from the brains of some inoculated rabbits, there was no evidence of multiplication of virus in their brains and passage of rabbit brain to rabbit did not produce encephalitis in subinoculated animals.

EMC, Col SK and MM viruses. Warren (45) records that EMC virus produces a mild infection in rabbits characterised by fever. He does not state whether any of these animals became paralysed, but all developed neutralizing antibodies in

their sera during convalescence.

Jungeblut et al (11, 12) reported that Col SK and MM viruses were non-pathogenic for rabbits, but do not report any tests for neutralizing antibody in the sera of inoculated animals. The failure to inject rabbits with Col SK virus was confirmed by Schultz et al. (45).

PATHOGENICITY FOR HAMSTERS.

ME virus. Hamsters exhibit symptoms similar to those found in mice after inoculation with ME virus. They are susceptible to inoculation by intracerebral or peripheral routes. Using small doses (10 to 33 mouse LD_{50}) of 3rd passage mouse-brain virus introduced by the intraperitoneal or intravenous routes the incubation period was 4 to 5 days. The hamsters in this group developed paralysis of one or more hind limbs and those which were not sacrificed remained paralysed up to the end of the observation period of 25 days. ME virus was recovered from the blood serum of all animals tested up to the 6th day and was found in the brains of all animals examined after the onset of symptoms. Serum tested from paralysed hamsters during the 3rd week after inoculation neutralized from 10,000 to 100,000 mouse LD_{50} of ME virus.

EMC, Col SK and MM Virus. The EMC and MM viruses are also pathogenic for hamsters (44, 45, 12) causing paralysis and death, depending on the size of the inoculum.

PATHOGENICITY FOR MONKEYS

Rhesus monkeys

ME virus. Although ME virus was first isolated from the brain and cord of a paralysed rhesus monkey, paralysis must be a rare manifestation of activity of this virus in rhesus monkeys. Only 2 out of 14 rhesus monkeys inoculated with ME virus have exhibited paralysis. Furthermore, rhesus 823, a naturally infected rhesus monkey, from which a strain of virus was isolated (p.15) showed no abnormality apart from pyrexia, and 8 rhesus monkeys from the outdoor runs of the laboratory at Entebbe, found to be immune as a result of naturally acquired infections with this agent, had no record of paralysis.

Both of the rhesus monkeys (692 and 600) in which paralysis was observed were inoculated with monkey-passage virus. The inoculum used for the former consisted of a pool of the unfiltered supernates of portions of the cortex, midbrain, pons, cerebellum, medulla and cord of the original paralysed rhesus monkey; 0.5 ml. of this pool was injected intracerebrally and 2 ml. subcutaneously. Rhesus 692 had fever (104.4°F.) on the 3rd day. It was bled daily for 10 days after inoculation, and the serum from the 4th to 7th days' bleedings produced paralysis and 100 per cent mortality when inoculated intracerebrally into groups of mice. The mortality ratio in mice inoculated with 3rd-day serum was 2/6. On the 9th day this monkey had slight weakness of the lower

limbs, and there was a suggestion of incoordination. By the 10th day, however, it again appeared normal, and serum taken at that time contained antibody to ME virus.

The other rhesus monkey (600) in which there was evidence of paralysis was inoculated intracerebrally with 0.1 ml. of the 8th day serum of rhesus 739, from which the human strain of this virus had been recovered (p.14). Rhesus 600 had pyrexia from the 2nd to the 8th day after inoculation (Fig. 1) and virus was demonstrated in its serum on the 2nd day; no other tests were made for circulating virus. From the 4th to 6th days rhesus 600 had anorexia, and it showed some weakness of the left leg on the 7th day. It made a complete recovery and developed neutralizing antibody to ME virus.

FIG. 1

With the exception of the above 2 monkeys and rhesus 739, all other inoculations of rhesus monkeys were made with mouse-brain virus and further studies of rhesus monkey to rhesus monkey-passage-virus are in progress.

Rhesus 739 was inoculated intracerebrally with 0.1 ml. of 2nd-day acute-phase serum from the human. On the 7th day after inoculation it had a slight elevation of temperature (103.8° F.) and on the following day a temperature of 105°F. It was bled on the 8th day, and its serum was subinoculated into another rhesus monkey (600), which has already been discussed. Rhesus 739 continued to show fever for 5 days, (Fig. 2) but no other manifestations of infection were noted.

Mice inoculated with the 8th-day serum of rhesus 739 showed 100 per cent mortality. Convalescent serum from this latter monkey contained neutralizing antibody to the virus in high titre.

FIG. 2

Eleven rhesus monkeys were inoculated with mouse-brain passage virus by the following routes: subcutaneous (4811 and 4816), intracerebral (621, F 76, F 85, F 86), intracerebral and subcutaneous (838), intraneural (690), intravenous (826), intramuscular (829) and by the subcutaneous inoculation of virus following an intracerebral inoculation of soluble starch (764). All of these monkeys had fever for varying periods from the 1st to 7th days after inoculation. Apart from 838 which showed marked hyper-excitability none of these monkeys showed any objective signs of involvement of the central nervous system. All of these monkeys were tested for circulating virus by the intracerebral inoculation of groups of mice. The titres of ME virus circulating in the blood stream of these animals in which daily tests were made are presented in Table 14. All of these rhesus monkeys developed neutralizing antibody to the agent as a result of the inoculations.

(TABLE 14.)

<u>EMC virus.</u> EMC virus is said to produce a variable response in rhesus monkeys (44), but the number of animals inoculated is not available. The disease is said to range from an acute febrile course followed by death within the first week, to

an inapparent infection characterised by transient fever and subsequent appearance of neutralizing antibody in the serum.

Col SK virus. The Col SK virus failed to bring about paralysis in rhesus monkeys in its earlier passages. After transmission through 70 mouse passages its virulence for monkeys was enhanced (49). Of 13 monkeys injected intracerebrally with mouse-brain virus, 1 monkey developed paresis and 5 monkeys passed through an encephalitic syndrome which proved fatal in 2 cases. In a further group of 14 monkeys receiving repeated intravenous injections of virus 2 animals developed partial or complete flaccid paralysis. In a more recent study by Schultz and White (45) 4 rhesus monkeys injected with large doses of mouse-passage virus failed to develop symptoms.

MM Virus. Although the original human material from patient MM contained a virus which produced poliomyelitis in the rhesus monkey, the MM virus which was derived from the hamster-passage brain was not pathogenic for monkeys (12). Jungeblut (50) states that with repeated passage in laboratory animals MM virus is now similar to Col SK virus in its pathogenicity for rhesus monkeys.

African Monkeys

ME Virus. (1) C. aethiops centralis Neuman (African grey monkeys) are more susceptible than rhesus monkeys to inoculation of this virus. Of 5 monkeys of this species inoculated with mouse-brain passage ME virus, paralysis was present in

all but one (594). This latter animal was inoculated intranasally with 1,112 mouse intracerebral LD_{50} of virus. No circulating virus was demonstrated in serum from 10 daily bleedings of this animal, and convalescent serum from it contained no neutralizing antibody to ME virus.

The data of the inoculation of the other monkeys is presented in Table 14. All these aethiops monkeys had elevations of temperature for 1 to 3 days between the 2nd and 7th days after inoculation. Aethiops 529 which was the only survivor in this group showed a marked disinclination to use its left arm on the 6th day after inoculation. Complete paralysis of this arm was not present, and by the 10th day there was complete recovery. Fourteenth-day serum from this monkey showed the presence of neutralizing antibody. Aethiops 732 was inoculated into the right thigh with ME virus immediately after an intracerebral inoculation of 1 ml. of soluble starch into the right cerebral cortex. On the 6th day after inoculation it was found to be dragging its right arm. Examination showed weakness of the right arm and of the pectoral and shoulder muscles of the right side. On the 7th day it was found dead. At autopsy some generalised congestion of the brain was observed. probably largely postmortem in nature. On the right side of the cerebral cortex a small congested area of about 2 mm. in diameter was noted, presumably the site of the starch trauma. Various portions of the brain and cord were removed and weighed.

Ten per cent suspensions were made of each portion with physiological saline solution as diluent, and groups of mice were inoculated with Seitz-filtrates of each suspension. The distribution of virus in the central nervous system of this monkey and in 2 other C. aethiops inoculated with virus is compared with that found in the original paralysed rhesus monkey in Table 15.

(TABLE 15)

Aethiops 733 was caged with 732 before and after the inoculation of the latter, in order to determine whether the virus might be transmitted to monkeys by contact. Aethiops 733 showed no abnormality throughout an observation period of 23 days. It was bled daily for the first 10 days. No circulating virus was demonstrated, and no circulating antibody was demonstrable at the conclusion of the experiment. Aethiops 627 was inoculated intravenously. The results of titrations of the serum of each day's bleeding presented in Table 14, give ample evidence of the multiplication of this virus in aethiops monkeys, which is greater than that found in rhesus monkeys given a comparable inoculation. On the morning of the 5th day aethiops 627 was found lying in its cage apparently unable to rise. Examination showed partial paralysis of the right lower limb. The right knee jerk was less marked than the left, which may have been exaggerated. The abdominal reflexes were absent, and the abdominal wall was flaccid. The gluteal muscles and muscles of the back were

weak. This monkey was sacrificed on the 5th day and the central nervous system was removed completely for histological examination. A small portion of the midbrain was suspended in saline solution and ME virus was demonstrated in this tissue by an intraperitoneal protection test in mice.

Aethiops 626 had anorexia from the 3rd day after inoculation and on the morning of the 5th day it exhibited partial paralysis of the left leg and was disinclined to use its left arm, although real paralysis in the latter could not be demonstrated with certainty. The animal was found dead on the morning of the 6th day. No gross abnormality was noted at autopsy. Groups of mice were inoculated with Seitz filtrates and with the unfiltered supernate of suspensions of various portions of the central nervous system. Virus was demonstrated in greatest concentrations in the lumbo-sacral and thoracic portions of the spinal cord, and in lesser concentrations in other areas of the central nervous system (Table 15).

(2) C. aethiops sabeus (African green monkeys) are less susceptible to the inoculation of virus. Of 4 monkeys (E395-E398) inoculated extraneurally with 0.5 ml of a 10 per cent suspension of 20th mouse-brain virus, 1 (E395) died on the 8th day after inoculation. None of the other animals showed any symptoms of infection. No specific lesions were demonstrated in the animal which died.

(3) Cercocebus fulginosus ("Sooty" mangabeys). 2 mangabeys were inoculated intrathalamically with 0.8 ml of 10 per cent mouse-brain virus (21st mouse-brain passage). One of the

animals (E382) was found dead on the 4th day after inoculation and the other (E385) was moribund on the 5th day, when it was sacrificed for histological material. Neither of these animals showed any signs or symptoms prior to the 4th and 5th days respectively. Of 2 mangabeys (E384 and E385) inoculated subcutaneously and intraperitoneally respectively, with 1 ml. of 10 per cent mouse-brain virus, E 384 was found dead on the 8th day after inoculation, E 385 remained well over an observation period of 21 days.

EMC. Col SK, MM Virus. No experiments have been made or are recorded of the susceptibility of African monkeys to these agents.

PATHOGENICITY FOR MAN

ME Virus

Case History:

The infection recorded took place in a laboratory worker who was living in the compound at Entebbe where ME virus was isolated from the rhesus monkeys, mongoose and T. (C) fusco-pennatus.

The source from which the patient acquired the infection is not known, and probably never will be. The patient had more laboratory contact with the virus than any of the other professional or technical staff of the Institute, yet some of the others also had considerable contact. In the period immediately preceding the illness caused by the virus, the patient was the only person in residence in the Institute compound. That there was "free" ME virus in the vicinity about

this time is attested by the isolation of the virus from a naturally infected rhesus monkey kept out of doors in the Instutute grounds, from mosquitoes caughtaround the monkey runs, and from a mongoose trapped in the compound. The infection may therefore have been contracted either naturally or in the laboratory. The point is, however, only of academic interest, since immunological studies have shown that man can acquire the infection naturally.

Clinical Features

Incubation Period. No definite information on the incubation period of the infection in man is available; but, if the infection was naturally acquired, and if, as seems most probable, it took place at Entebbe, the maximal incubation period was probably 9 days, because before that the patient had been absent from Entebbe for 11 days. If the infection was contracted in the laboratory the incubation period was probably 5 to 8 days estimated from the dates on which the patient was handling ME virus before the beginning of his illness.

Onset. On November 22, 1946, he felt slightly "out of sorts" physically, but towards evening he became mentally very alert. He experienced slight shivering at night. Next day he awoke with a very severe headache, of a "bursting" type, which was aggravated by coughing or by quick movements. Later the headache became so violent that he hesitated to cough or to strain in any way. He had no fever and proceeded on safari, driving 240 miles. On the journey he took repeated doses of aspirin but these did not relieve the headache, though subse-

quently its intensity was somewhat diminished by alcohol. Course. On the morning of November 24 the patient's headache was very severe. About noon he began to shiver, could not walk about, and felt better lying in a dark room. He took some 'Veganin' and 2 gm. of sulphadiazine.

He was examined by Dr. A. M. Best (M. O. Fort Portal) on November 24, when his general condition was good, though his temperature was 101.8 F (Fig. 3). His pulse-rate was 84 per min. and the pulse was of good volume. His face was flushed and his conjunctivae injected. His pupils were equal and reacted briskly to light and accommodation. He had some hyperaesthesia over the scalp and forehead. By the evening his headache was worse and he was delirious, with irritability, restlessness, and delusions. He vomited three times between 7 and 10 p.m. Repeated blood slides were negative for malaria and other parasites. The patient was given a tablet of 'Veganin' and morphine gr. 1/4 and had four hours of heavy sleep. On November 25 his headache was intense, generalised, and "bursting" in type, and he had severe photophobia. His tongue was moist and moderately furred. There was definite rigidity of the neck on flexing the chin on the chest. All arm and leg reflexes were sluggish. The plantar reflex was flexor, and the abdominal reflexes were brisk. Hyperaesthesia of forehead and scalp was present. No abnormality of the cranial nerves was found, but the optic discs were blurred. Blood slides were again negative for parasites. The total white-cell count was 7300 per c.mm. (polymorphs 65%,

lymphocytes 33%, monocytes 2%). The patient was still very restless and mildly delirious and his temperature was 102.8°F. He had a poor night in spite of medication with morphine and bromides, but by next morning (Nov. 26) his temperature had fallen to 99.4°F, his headache had become less severe, and he was more relaxed. Photophobia, neck-rigidity, mild oedema of the optic discs, and hyperaesthesia of the head were still present.

Next day all signs and symptoms diminished, and the patient's temperature was normal, but in the evening he had tingling of the right forearm and hand, with some numbness. No abnormality of sensation or motor function was detected. The white-cell count was 8400 per c.mm. (polymorphs 58%, lymphocytes 39%, monocytes 3%).

By the 28th, the patient had further improved. He now had only occasional twinges of headache, and his temperature was still normal. He was allowed to get up. Next day he did not complain of any symptoms apart from paraesthesia of the right forearm. On November 30 he discovered that he was deaf in the right ear and had some weakness of the upper fibres of the trapezius muscle on the right side. The deafness was subsequently shown to be of nerve type.

During the first fortnight of convalescence there was some weakness of the muscles of deglutition, but this and the weakness of the trapezius muscle disappeared within about three weeks. Convalescence was uneventful, and the only sequela was slight residual nerve deafness on the right side.

The isolation of virus and demonstration of the development of neutralizing antibody during convalescence has already been discussed (p.13).

Human Immunity Surveys

No specific studies have been made for the purpose of studying the incidence of human immunity, as indicated by demonstrable circulating antibody to ME virus, in Africa. A few sera collected for yellow fever immunity tests have been examined for their neutralizing effect on this virus. In testing these sera 0.25 ml. portions of a 1 in 100 dilution of mouse-brain virus were added to 0.5 ml. of the serum under test. The virus-serum mixtures were allowed to stand at room temperature for periods of 20 to 30 minutes before inoculation into mice. The virus suspensions employed in the tests were titrated from 10^{-2} to 10^{-6} against undiluted normal sera. An immune control was set up in which immune human or monkey serum was diluted from 1 in 4 to 1 in 256, and 0.25 ml. of 1 in 100 virus was added to undiluted and to each dilution of immune serum. The test dose of virus was usually in the region of 1,000 LD_{50} per mouse. In all tests early-passage virus was employed. Mice were observed for 10 days. If 5 or more mice survived, the serum was regarded as protective. If 2 or less mice survived, the serum was regarded as non-protective. If more than 2 but less than 5 mice survived, the test was considered inconclusive and the serum was retested, usually against 2 or more dilutions of virus. In a few of the cases in which serum-virus mixtures produced 100 per cent

mortality, the mice showed a survival time longer than the average; these sera were also retested against higher dilutions of virus. In all cases the occasional serum which gave an inconclusive result was negative on retest. All sera in which a clearcut positive result was obtained remained positive on retest.

Sera from 237 persons have been tested, and 3 have been found to be immune: 1 from the patient whose case is described here; and 2 from children aged 4 and 6 years, both living on the edge of the Budongo Forest in Siba village, Western Province, Uganda. The results of the protection tests, excluding the case described, were as follows:

Area where sera were collected	No. tested	No. immune
Uganda:		
Bwamba County	29	0
Budongo Forest	61	2 (3.3%)
Toro District	20	0
Kalinzu Forest	28	0
Various areas	30	0
Northern Rhodesia	39	0
Tanganyika	9	0
Miscellaneous	20	0
Total	236	2 (0.8%)

Dr. C. R. Rainsford, senior medical officer, Western Province, Uganda, kindly examined the two children who had protective sera and reported that they had no neurological symptoms. It is planned to carry out more extensive studies on the distribution of human immunity to ME virus in Africa and elsewhere.

EMC virus

Case Histories

During the winter of 1946 numerous cases of "3 Day Fever"

occurred among army personnel stationed at or near Manila, PI. The onset of the disease was sudden with a moderately high fever, reaching 104°F in some instances, and severe headaches. The fever lasted for 2-3 days and was accompanied by stiff neck, a positive Kernig's sign, hyperactive deep reflexes and pharyngitis. An occasional patient was comatose on admission to hospital. The only notable laboratory finding was a pleocytosis of from 50-500 cells principally lymphocytes. There were no sequelae and no signs of cardiac disease were observed.

Human Immunity Surveys

Sera from 44 of the soldiers with this "3 day fever" were available for study. 17 of the 44 had appreciable amounts of specific neutralizing antibody for the EMC virus. Rising antibody titres were demonstrated during convalescense in 3 or 4 patients from whom several samples of sera were available (51).

Studies of the presence of this disease have been made on a limited scale in U.S.A. Warren (52) has demonstrated neutralizing antibody in 9 of 300 sera tested. 5 of these 9 sera were from cases diagnosed as abortive or mild poliomyelitis, 3 as aseptic meningitis while 1 sera was from a patient with upper respiratory symptoms and headache. In all these cases the sera neutralized more than 500 LD_{50} of EMC or ME virus.

MM and Col SK Virus

MM virus was said to have been isolated from a fatal

case diagnosed as poliomyelitis (12). As has been pointed out, no studies have been done to determine whether or not any immunological relationship existed between the monkey pathogenic agent in the original human material and the virus derived from the hamster brain.

The immunological differences of the Col SK virus and the SK virus isolated from the human case of poliomelitis have already been discussed. Jungeblut and his associates have not recorded any serological studies with Col SK or MM viruses and human sera which are not equivocal. From a limited study of the neutralization of Col SK and MM viruses it is clear that human sera which neutralize ME virus show equal neutralization of Col SK and MM viruses.

ANTIBODY STUDIES AMONG ANIMALS

Monkeys

ME virus. Tests made on sera from 23 rhesus monkeys from the outdoor runs at Entebbe showed that 8 (35 per cent) were immune to ME virus. (In none of these monkeys was any paralysis recorded.) In the case of 2 of them, preinfection sera were available for comparison with the later sera found to contain antibody. The results of the protection tests indicated that immunity in these 2 monkeys had been naturally acquired on the Institute compound within the year 1947. As in the case of rhesus monkeys, natural infection has occurred among the normal stock of Cercopithecus aethiops centralis Neumann monkeys. Of 6 unused C. aethiops monkeys, 1 was found to be immune, but to date none of them has been found paralysed. As has already been said, monkeys which have survived experi-

mental inoculations of ME virus or have experienced natural infections with this virus have developed high levels of circulating antibody. Sera from 18 wild monkeys from western and central Uganda, which had been obtained for yellow fever protection tests, were tested against ME virus, but none of them neutralized this virus.

Col SK and MM viruses.

The results of antibody studies using monkey sera and Col SK and MM viruses presented by Jungeblut, et al (49) are irregular.

Mongooses

ME virus. No tests have as yet been made with the sera of other wild animals except those of 4 mongooses (of unidentified species) trapped in the compound of the Yellow Fever Institute, Entebbe. One of these sera neutralized more than 1,000 mouse LD_{50} of ME virus.

Rats and Other Rodents

EMC virus. Sera from over 400 wild rats caught in various states of USA have been tested for antibody to EMC virus. 18/ per cent of these sera had antibody to this agent with neutralization indices between 1,000 and 100,000 (54). Other rodents such as rabbits and chipmunks caught in "positive areas" were all devoid of antibody. It was found that sera protecting mice against EMC virus showed a similar degree of protection with ME and MM viruses.

PHYSICAL PROPERTIES

Filterability

ME, EMC and Col SK viruses. There was no appreciable decline in the titres of mouse-brain virus after passage through Berkefeld V, N, or W filters or through Seitz E.K.pads. Similar findings are reported for Col SK(11) and EMC viruses (44).

Size

ME virus. Estimations of the size of ME virus were made by the method of Elford (55). Several experiments were done, of which the following is a typical example. Brains were taken from 10 mice paralysed as a result of inoculation with 4th-mouse-brain passage ME virus. A 10 per cent suspension of these brains was made in broth of pH 7.4. This suspension was centrifuged at 1,500 r.p.m. for 15 minutes and the supernate was passed through a Seitz E.K. pad. The Seitz filtrate was then put through a collodion membrane of 455 mμ. pore size. This latter filtrate was then passed through filters of 50, 45, 37, 30, and 22 mμ. size, at 8 pounds pressure. These filtrates were each tested by the intracerebral inoculation of 6 mice. No mice died as a result of inoculation with the 30 m . and 22 mμ. filtrates. There was 100 per cent mortality of mice inoculated with the other filtrates. From these experiments, the particle size of ME virus is estimated at 10 to 15 mμ. which is the estimated size of the virus of poliomyelitis (56, 57).

EMC, Col SK and MM viruses. The virus of EMC passed gradacol membranes of an average pore size of 30 mμ. Thus the diameter of the particle probably lies in the range of 8 to 15 mμ. No estimate of the size of Col SK virus is available but Jungeblut et al (11) state that the ease with which it filtered through a W. Berkefeld filter suggests a particle size consistent with the known dimensions of poliomyelitis virus. The size of MM virus appears to be in the same range of magnitude as that of poliomyelitis virus (12).

POTENCY OF DESICCATED VIRUS

ME virus. Several lots of mouse-brain containing ME virus were preserved by desiccation. The material to be desiccated was suspended in whole normal serum, the suspension was centrifuged and the supernate was distributed in Pyrex ampoules. This material was frozen and dried on a Flosdorf-Mudd glass manifold, and the ampoules containing the dried virus were sealed with an acetylene flame while the vacuum pump was in operation. The dried material was tested periodically for potency. Although there was considerable loss of potency in the dried material after 10 days of storage, this material still contained virus after 3 years in a refrigerator at approximately $4^{\circ}C$.

EMC and Col SK viruses. EMC virus is quite stable in the lyophilised state. Preliminary experiments with Col SK virus suggested some instability on desiccation (11) but no titrations on stored material are available.

POTENCY OF VIRUS PRESERVED IN GLYCERINE

ME virus. Several lots of infected mouse brains were placed in a mixture of equal parts of glycerine and normal saline and were stored in a refrigerator. Titrations were made of 5 lots of virus thus preserved for periods of 9 to 10 months, and in only one case was the titre less than $10^{-5.5}$. The titre of virus in fresh mouse-brain has seldom been under 10^{-6}. It may, therefore, be stated that up to 9 months there is relatively little loss in the virus content of mouse-brains preserved in 50 per cent glycerine. The diminution in virus titre is much less in mouse brain thus preserved than in desiccated mouse-brain.

EMC and Col SK viruses. EMC and Col SK are both said to be well-preserved in 50 per cent glycerine (44, 11).

SURVIVAL OF VIRUS IN SUSPENSIONS

ME virus. Experiments were done to discover the effect of sodium chloride on ME virus. Brains of two paralysed mice of the 11th passage were suspended in distilled water to make a 10 per cent suspension. Serial decimal dilutions of this suspension were prepared with physiological saline solution and serum-saline solution. After the serial dilutions has been made, 0.5 ml. of normal serum was added to 0.25 ml. of the serum-saline dilution. The mixtures were incubated for 1 hour at 37°C., and groups of mice were then inoculated intraperitoneally with 0.06 ml. of each mixture. The results of the experiment showed that the titre of virus in normal serum was $10^{-6.6}$ and the titre in the saline mixture $10^{-5.8}$,

indicating that saline had only moderate effect, if any, on the potency of the virus[1].

Experiments were then carried out to compare the survival time of virus in saline and in serum-saline solutions at refrigerator, incubator, and room temperature. In the first experiment, brains of 40 paralysed mice of the 56th passage were weighed and then ground up with 7 volumes of distilled water. To 7 volumes of the Seitz-filtered virus suspension were added 1 volume of 8.5 per cent saline and 1 volume of normal monkey serum. To another 7 volumes of the suspension 1 volume of 8.5 per cent saline and 1 volume of distilled water were added. Each batch was then distributed into ampoules in 51 lots. The ampoules were sealed and one third of each lot was kept in the incubator (37.5° C.), one third at room temperature (21° to 25° C.), and the remainder in the refrigerator (2° to 10° C.).

Mice were inoculated at various intervals of time with serial dilutions of each suspension. No virus could be demonstrated on the 25th day in the suspensions in saline stored in the incubator (37.5° C.). No end point was obtained with virus in serum-saline suspensions stored at this temperature, but the period of survival was more than 33 days (Table 16).

(TABLE 16)

The titre of virus suspended in saline solution

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1. It has been calculated that in virus titrations the difference in titres must be greater than 0.8 log. to be significant.

and stored at room temperature had dropped from $10^{-6.6}$ (on day 0) to $10^{-4.0}$ after 31 days. The titre of virus in serum-saline solution on the 31st day of storage at room temperature, was $10^{-5.0}$. By the 102nd day of storage at this temperature, virus was still present in the serum-saline suspension (titre $10^{-2.8}$). Unfortunately, all the ampoules of virus suspended in saline solution were used by this time and no comparison with the saline suspension is available.

At refrigerator temperature there was more virus in the serum-saline suspension (titre $10^{-3.0}$) than in the saline suspension (titre $10^{-2.9}$) on the 90th day; on the 117th day of storage titres of $10^{-2.0}$ and $10^{-3.2}$ respectively were obtained.

In all experiments the loss of virus activiy in the serum-free medium has been greatest during the 1st week of storage of the suspensions.

EMC, Col SK and MM virus, No studies of the stability of these agents has been published.

THERMAL DEATH POINT

ME virus. Suspensions of virus containing 10 per cent (by weight) of infective mouse brains were made in 10 per cent monkey serum in physiological saline solution. The suspensions were centrifuged and passed through Seitz E.K. pads, and the filtrates distributed in 0.5 ml. or in 1 ml. quantities into glass ampoules. After sealing, the ampoules were completely immersed in water baths maintained at the various temperatures required for the test. Ampoules were

removed from each flask after the desired time intervals and groups of mice were inoculated intracerebrally with 0.03 ml. of the contents of each ampoule.

It was found that when ME mouse-brain virus was suspended in 10 per cent serum-saline, it withstood heating at 94° C. for 20 minutes, and that in that diluent it could even withstand heating at 98° C. for 5 minutes. All virus particles were destroyed when suspensions of mouse-brain virus were heated at 96° C. for 20 minutes, at 98° C. for 10 minutes or at 100° C. for 5 minutes. These observations on the marked thermo-stability of suspensions of this virus in serum-saline solutions were confirmed by many experiments.

All virus particles were destroyed, however, when a 10 per cent Seitz filtrate of mouse-brain virus in a serum-free diluent was heated at 60° C. for 30 minutes.

Using a similar technique to that described above, tests were made of the thermo-stability of ME mouse-brain virus suspended in a diluent consisting of 0.1 per cent bovine albumin in buffered saline solution (42). All mice inoculated with suspensions of ME virus heated at 60° C., 70° C. and 80° C. for 30 minutes remained well over an observation period of 10 days. The groups of mice inoculated with suspensions of virus heated at 56° C. for 2 and 3 hours had mortality ratios of 6/6 and 5/6 respectively.

An experiment was done to make a direct comparison under identical conditions of the thermo-stability of mouse-brain virus suspended in (a) 10 per cent serum-saline and (b) 0.1

per cent bovine albumin in saline solution. A 20 per cent suspension by weight of infective mouse brains was made in saline solution buffered at pH 7.4. After centrifuging at 2,000 r.p.m. for 20 minutes the supernate was passed through a Seitz E.K. pad. This filtrate was divided equally. To one portion was added an equal quantity of 20 per cent monkey serum in saline solution and to the other an equal quantity of 0.2 per cent bovine albumin in saline solution. The final concentrations were 10 per cent mouse-brain virus in (a) 10 per cent serum-saline solution and (b) 0.1 per cent bovine albumin in saline solution. These suspensions were heated as already described at 60° C., 70° C. and 80° C. for 30 minutes. The mortality ratios of mice inoculated with the contents of the ampoules are presented in Table 17.

(TABLE 17)

It may be seen from Table 17 that the thermo-stability of ME virus varies very markedly when the virus is suspended in a diluent containing serum and in a serum-free medium. One hypothesis, (from this experiment and from the results of other experiments cited above), might be that when the virus is heated in a suspension containing 10 per cent serum, some of the virus particles become encased in coagulated serum proteins which in some way insulate the virus and prevent its destruction by heat. Another possibility is that there may be a reversal of the denaturation of the protein of the virus particle which presumably occurs on heating. Such a reversal of denaturation has been observed with certain pure proteins (58).

EMC and Col SK viruses. Col SK virus is said to be completely destroyed by heat for 1 hour at 60° C. EMC virus was inactivated by a temperature of 60° C. for 30 minutes but not by 56° C. for one-half hour (44). Within the variations of the experimental technique there is, therefore, general agreement on the thermo-stability of the viruses of this group.

SUSCEPTIBILITY TO CHEMICALS

ME virus. A 10 per cent suspension of mouse-brain virus of the 95th passage was made and put through a Seitz E.K. filter. The filtrate was diluted with saline to 1 in 100 and distributed in test tubes in 5 ml. quantities. Equal volumes of the chemicals to be tested were added to give the final concentrations shown in Table 18. The tests were made at room temperature (approximately 20° to 24° C.). The mixtures were inoculated intraperitoneally into groups of mice in 0.06 ml. quantities after the filtrates and the chemicals had been in contact for 12, and 30 minutes, and 1, 6, 24, and 72 hours.

(TABLE 18)

It may be seen from Table 18 that potassium permanganate in a concentration of 1 in 200 inactivated the virus within 12 minutes; at a concentration of 1 in 2,000 there was some loss of virus activity after 12 minutes of contact, but virus could still be demonstrated in this mixture up to at least 60 minutes. Active virus could be demonstrated in a 1 per cent hydrogen peroxide mixture after 24 hours of contact and in a 1 per cent phenol mixture after 6 hours. In a concentration of 0.5 to 1 per cent formalin the virus retained its activity

beyond 60 minutes, and after contact with alcohol (1 to 5 per cent) and ether (50 per cent) for 72 hours it still showed marked activity.

Col SK virus. This agent was shown to resist phenol up to 1 per cent concentration and tolerate acid to pH 4, but no time periods are recorded (11). It was destroyed by ultra-violet light after exposure for 1 minute but the technique of the experiment is not given (11).

CULTIVATION IN EMBRYONATED EGGS

ME virus. This agent was readily adapted to chick embryos and the distribution of the virus in the embryo, the rate of growth and the susceptibility of the embryo to infection by various routes of inoculation were studied.

The eggs used were of White Leghorn stock and were inoculated after 10 to 11 days' incubation at 38°C. After inoculation eggs were incubated at 35°C. The techniques of inoculation were as follows: (1) Chorioallantoic route. The method employed was essentially that described by Burnet (59) except that an oval window was cut in the shell and the oval portion of shell flipped up with a needle. This is found preferable to cutting a triangular opening and presents less danger of damaging the chorioallantoic membrane on removing the shell. After dropping the membrane by the usual technique the hole in the shell was sealed with "scotch tape". (2) Amniotic route. An opening was cut over the air sac and the shell membrane clarified with a few drops of "Bayol" mineral oil. The amniotic sac could then be visualized over an egg-candler

and inoculated directly. The openings over the air sac were sealed by affixing the cap of a screw-top bottle over the hole with paraffin wax. This method (which is now employed in the International Health Division laboratory of the Rockefeller Foundation) does not involve the trauma to the membranes which occurs with other techniques and has a very low non-specific mortality rate of embryos. (3) Yolk sac route. A procedure similar to that employed for amniotic inoculations was used in which the yolk sac was visualized through the cleared shell membrane. (4) Allantoic route. Eggs were inoculated through a small groove cut on the shell over a vessel-free portion of the chorioallantoic membrane: a hole was also drilled into the air sac. Both holes were sealed with paraffin wax after inoculation. Groups of 4 to 8 eggs were inoculated with each dilution of virus employed. The diluent employed in all egg experiments was 0.2% bovine albumin in buffered saline solution (42) containing 200 units of penicillin per ml.

Preliminary experiments showed that chick embryos were susceptible to inoculations of ME virus by all of inoculation. Inoculations of 46- 4,650 LD_{50} of mouse-brain virus by the amniotic or yolk sac route caused death of the embryos by the 3rd day after inoculation. Dead embryos showed extensive haemorrhages. When the allantoic or the chorioallantoic route was employed no abnormality was noted by the 4th day on first egg passage. After one passage in eggs the chick embryo was irregularly susceptible to inoculation of egg-passage virus

by these latter routes. Further studies are required on this problem but it may be, from the irregular nature of the results, that it is only when the membranes have been traumatized as a result of manipulation, that embryos are susceptible to chorio-allantoic or allantoic inoculation of ME virus.

Comparison of titre of 1st egg-passage virus in eggs and mice.

A comparison was made of the titre of virus in eggs and in mice. 3 dead embryos of the 1st egg-passage were ground in a chilled Waring blender for 1 minute to make a 10 per cent suspension. This suspension was centrifuged at 1,500 r.p.m. and serial decimal dilutions were made with the supernate. Eggs were inoculated by the amniotic route and mice intracerebrally with these serial dilutions. The LD_{50} for chick embryos has $10^{-5.3}$ and for mice $10^{-5.2}$. Repeated titrations showed that the end point of infectivity was equally reproducible when chick embryo-passage virus was inoculated amniotically into chick embryos or intracerebrally into mice.

Rate of multiplication of virus in the embryo.

16 eggs were each inoculated amniotically with 0.2 ml. of 10^{-4} dilution of 1st egg-passage virus. The inoculum was prepared from the unfiltered supernate of a suspension of dead embryos which had been centrifuged as a 20 per cent suspension at 1,500 r.p.m. for 15 minutes. The embryos of 3 inoculated eggs were harvested after 24, 48, and 72 house of incubation. By 72 hours all embryos except 1 were dead and haemorrhagic. The harvested embryos were ground in a Waring blender for 1

minute to make 20 per cent suspensions. These suspensions were shell frozen and stored at -70° C. Subsequently all suspensions were centrifuged simultaneously and serial decimal dilutions were made with each supernate and tested by intracerebral inoculation of mice. The results of these titrations are presented in Table 19.

(TABLE 19)

A second experiment (Table 19) was made in which 16 eggs were inoculated amniotically with 84 chick LD_{50} of 2nd egg-passage virus prepared as above from a suspension of embryos. 3 live embryos were harvested at 4, 21 and 48 hours after inoculation and titration of the virus content of the embryos was also made. The rate of multiplication of virus as indicated by both these experiments is presented graphically in Figure 4.

It may be seen that there is a lag in the multiplication of the virus up to at least 4 hours after inoculation. Multiplication of virus then takes place rapidly up to 48 hours. After this there is little appreciable change in the titre till the death of all the embryos, which in this experiment occurred within 96 hours after inoculation.

Rate of multiplication of virus in chick embryos after inoculation by various routes.

Groups each of 12 eggs were inoculated with 84 chick LD_{50} of chick-embryo virus by the amniotic, allantoic, yolk sac and chorio-allantoic routes. Whole embryos, yolk sacs and allantoic fluids from 3 of each group of eggs were harvested after 24 and 48 hours and separately pooled. The

embryos freed of membranes, and the yolk sacs were washed in sterile saline solution, drained and ground in a Waring blender to make 20 per cent suspensions. These suspensions and the allantoic fluids were stored at -70° C. Subsequently serial decimal dilutions were made with the supernates of the embryos and yolk sacs and with allantoic fluids. These dilutions were tested for virus by the intracerebral inoculation of mice. The results of this experiment are presented in Table 20.

(TABLE 20)

It may be seen that there is little difference in the titre of virus in the embryo at 24 and 48 hours after amniotic or yolk sac inoculation. After inoculation by the chorio-allantoic route there was a considerable lag before virus multiplication in the embryo was appreciable. After allantoic inoculation the titre of virus in the embryo was less than $10^{-1.5}$ 48 hours after inoculation.

When inoculations were made by the yolk sac or amniotic route the yolk sac contained large quantities of virus by 48 hours. After inoculation by the chorio-allantoic route the yolk sac and the whole embryo contained less virus than when inoculations were made by the yolk sac or amniotic route. It was possible to demonstrate only a small quantity of virus in the yolk sac 48 hours after inoculation by the allantoic route.

Titrations of the allantoic fluid harvested 24 hours after inoculation revealed only traces (less than $10^{-1.5}$) of virus after inoculation by one or other of the routes

employed. It may be, that larger quantities of virus could have been demonstrated in the allantoic fluids at 48 hours after inoculation. These studies of the multiplication of virus in the embryonated egg are essentially preliminary in nature.

EMC virus. Warren (44) reports that EMC virus caused death of embryos within 72 or 96 hours after inoculation, that emulsions from infected embryos were infectious at dilutions of 10^{-7} while allantoic fluids possess titre of 10^{-4} to 10^{-5} when titred intracerebrally in mice.

Col SK virus. Schultz and Enright (60) carried the Col SK virus through 30 egg passages. The LD_{50} for mice in 15th, 21st, 24th and 30th egg passages was 10^{-7} based on titrations of Chamberland L_3 filtrates of 1 per cent suspensions of whole embryo tissue. They found this agent to be widely distributed in the infected embryo and it was easily transmitted from egg to egg by different routes. Embryos from 5 to about 14 days of age seem to serve equally well for the propagation of this agent but 16 day old embryos failed to support growth. Cultivation of this agent in embryonated eggs was also made by Powell and Jamieson (61), who note that like ME virus the virus content of periembryonic fluids was less than 10^{-2} while the titre of whole embryos was in the region of 10^{-7}.

MM virus. This agent was cultivated in the chick embryo by Enright and Schultz (62) and by Powell and Jamieson (61). In all respects this strain was similar to that of the Col SK virus with regard to egg pathogenicity.

PATHOLOGICAL LESIONS INDUCED BY ME VIRUS

Central nervous system lesions

ME virus. Lesions produced by ME virus have been studied in mice, guinea pigs, hamsters and monkeys. No lesions were demonstrated in the central nervous system of inoculated rabbits.

Mice. Material was obtainable from mice in the preparalytic stage and in the paralytic stage, in both early and late passage infections. In the brains of mice which were examined before the onset of objective signs of infection there was generally a certain amount of congestion, particularly of the pia. Damage to the nerve cells of all portions of the brain, with the exception of the Purkinje cells, was in evidence. In many neurones a splitting of the nucleolus into its basophilic and eosinophilic constituents was present. The greatest damage in the brains of these mice was present in the hippocampus; this was constantly most marked in the anterior part of the outer molecular layer. There was little evidence of cellular infiltration either in the brain or in the cord. In the cord, as in the brain, there was damage to the ganglion cells.

In the central nervous system of mice which had become paralysed within 48 hours of inoculation there was marked necrosis of the hippocampus. In some cases the outer molecular layer of the hippocampus contained a section of completely necrotic cells, and in most cases this necrotic section was quite clearly demarcated from cells in which minimal cellular

changes were present. In all mouse brains examined these areas of necrosis have been confined to the anterior portion of the molecular layer of the hippocampus. In such necrotic areas very little cellular reaction was present and there was only a small amount of infiltration of the adjacent white matter. A variable amount of neuronal damage was present in other portions of the brain, particularly in the base of the brain. Throughout the cord there was evidence of nerve cell damage and a moderate but variable amount of cellular infiltration of the grey matter.

In mice which had been paralysed for several days the hippocampal lesions were often less marked. Areas of focal necrosis were present in various parts of the brain in which focal accumulation of glial nuclei were present. Compared with the mice in which there had been a rapid onset of paralysis, these mice showed a much greater diffuse cellular infiltration and more marked cuffing of the vessels, particularly in the spinal cord. Generally the cuffing was most evident in the lumbar portion of the cord. Owing to the presence of a certain amount of nonspecific necrosis in the Purkinje cells of nearly all normal mouse brains, probably due to the technical methods employed, the extent of damage produced in these cells by ME virus was not readily estimated; it was minimal, however, and was seen only in the brains of mice which had been paralysed for 5 or more days. In these mice the Purkinje cells showed eosinophilic nuclear changes, loss of their processes and rounding up of the cell body.

The greatest amount of cellular infiltration was found in mice which had been paralysed for the longest periods. In such animals there was very marked cuffing of vessels both in the brain and spinal cord, and there were scattered areas of focal necrosis. In some parts of the cord there were almost no nerve cells, and in some mice there was a diffuse infiltration of the white as well as the grey matter.

Guinea pigs. The occurrence of pathological changes in the central nervous system of guinea pigs was dependent on whether the infection was of an acute or of a more chronic nature. In guinea pig 337 which was sacrificed on the 2nd day after inoculation, the most severe damage was present in the hippocampus, in which there were large areas of complete necrosis in the molecular layer. There was little leucocytic reaction in these necrotic areas. The neurons throughout the brain and cord showed pathological changes. The most characteristic changes were in the muclei of the neurons. In no place was there the complete margination of nuclear chromatin characteristic of certain viral diseases. The nuclei, however, showed a partial margination of chromatin, while the rest of the nucleoplasm was disintegrated into an eosinophilic nuclear dust. The nucleoli of the damaged nerve cells were eccentric and could often be seen to have disintegrated into a dot of eosinophilic and a dot of basophilic material. Occasionally all that was left of the nucleus was the nuclear membrane to which the disintegrating basophilic and eosinophilic constituents of the nucleolus were adhering.

There was little cellular infiltration of the brain as compared with the cord, where the perivascular cuffing was marked. In guinea pig 337 there were quite a number of polymorphs in the region of necrotic cells and around the blood vessels. These accumulations of polymorphs were not perivascular but were arranged in foci at one side of the vessels. In another guinea pig (338), which died on the 2nd day after inoculation, there was no necrosis seen in the hippocampus, but very severe ganglion cell damage was present in the midbrain and pons. Nerve cell damage was also present in the medulla and throughout the cord. In guinea pig 341, which was sacrificed on the 9th day after inoculation, there was a minimal amount of damage in the brain. In the cord of this animal, however, there were areas, particularly in the lumbar region, where destruction of the ganglion cells was complete. Throughout the cord there was marked infiltration with small round lymphocyte-like cells, and with mononuclear cells ingesting the necrotic material. In none of the material from guinea pigs was there evidence of any meningeal or ependymal reaction.

In Monkeys.

The pathological material from monkeys has been limited. This is partly due to the small number of these animals which died or were sacrificed after inoculations of virus. The chief limitation was, however, that only in the later studies were perfusion techniques employed and adequate control material to hand.

In the spinal cord of the paralysed rhesus UR 22 from which the virus was first isolated, there was marked destruction of the ganglion cells, particularly in the anterior horns of the lumbar and cervical regions. All stages of ganglion cell damage were seen, from mild chromatolysis to complete destruction of the neuron. Many of the dying and dead ganglion cells were surrounded and invaded by macrophages. In the medulla, pons, midbrain and cerebral cortex there was also evidence of diffuse chromatolysis and generalised cellular infiltration of the grey matter. Cuffing of the vessels was moderate in the cord, but less marked in the brain and brain stem.

Similar lesions were found, particularly in the upper cervical and lumbar region in the cord of aethiops 732. Neuronal damage was most pronounced in the lumbar region. In this case there was little cuffing of the vessels, but all stages of degeneration of ganglion cells up to complete necrosis were present. Various portions of the brain of aethiops 732 were examined. Apart from a certain amount of cellular infiltration, the cerebellum appeared normal and the Purkinje cells showed no specific changes. In the cortex there was a fairly diffuse and widespread chromatolysis of nerve cells, but very little evidence of perivascular cuffing. There was marked chromatolysis of neurons in the pons but, as in the rest of the brain of this monkey, there was little evidence of perivascular infiltration.

In aethiops 626 there was evidence of chromatolysis and

cellular infiltrations in the cord, but there were minimal changes in the cerebrum, midbrain and pons. No cerebellar lesions were found.

For all the above material fixation was done with Zenker's fluid and the tissues were embedded in paraffin. Sections were cut at approximately 5μ. and stained with haemotoxylene-eosin. The limitation of this technique for the examination of central nervous system tissue is considerable. For the more recent studies, (which are still in progress) the technique employed at the Poliomyelitis Research Center of the Johns Hopkins University School of Hygiene and Public Health has been employed.

Briefly, animals are anaesthetised with ether and the thoracic cavity is opened. A canula is tied into the aorta through an incision in the left ventricle and the right ventricle opened. Perfusion with about 30-50 ml. of physiological saline solution is followed by perfusion with 1 to 2 litres of 10 per cent formol containing 1 per cent acetic acid. The perfused carcass may then be refrigerated for 24-48 hours and the CNS is then removed in toto. Further fixation in 5% formalin precedes embedding in celloidin and the cutting of serial sections of 30-60μ. and staining with gallocyanin (63).

The CNS of 4 mangabeys (Cercocebus fulginosus) inoculated with ME virus were prepared in this way. No lesions were found in the central nervous system of the 2 animals which were inoculated by peripheral routes. In the 2 animals inoculated intracerebrally the lesions in the central nervous system were similar in distribution and nature. There was a mild meningeal reaction over the entire surface of the brain. The choroid plexus of the ventricles was greatly infiltrated with round cells. This choroiditis was most marked in the

choroid plexus of the lateral ventricles. There was cuffing of the vessels throughout the brain-stem, particularly marked beneath the brain surface and around the ventricular system. In making comparisons with normal mangabey brains there was little evidence of chromatolysis of the neurons. It should be noted that the neurons of the normal mangabey have a much more chromatolytic appearance than those found in normal rhesus monkey or in human brains.

The most marked lesions were found in the corpus callosum where there were considerable degenerative changes, with the presence of blood pigment. It could not be established conclusively that these degenerative changes in the white matter were not related to the site of inoculation, but no association with the needle tracts could be established, and it seemed most unlikely that the corpus callosum was inoculated by chance in both of the animals. Further studies are in process to clarify this point. In the cords of both the mangabeys there was marked neuronal destruction and infiltration in those portions of the lumbar region of the cord (L 1) which were examined.

Sections of the cord and brain were also stained with Haematoxylene and Azure II. Inclusion bodies similar to those found in poliomyelitis infections were demonstrated. No lesions were observed in other portions of the cord. It would appear from these preliminary histological studies that the lumbar portion of the cord is more sensitive to the action of this virus than other portions of the CNS. It is of interest

to recall in this connection that the largest amount of virus, irrespective of the route of inoculation, has usually been found in the lumbar portion of the cord of sacrificed animals. (p.51)

EMC virus. No adequate studies made of the central nervous system lesions induced by EMC virus are available.

Col SK and MM viruses. Jungeblut et al (11) have stressed the similarity between the lesions induced by Col SK and MM viruses and the virus of poliomyelitis. While both these agents are associated with lesions in the anterior horn cells of the spinal cords of rodents, lesions are also widespread in the remainder of the central nervous system.

Other Lesions

ME virus. In the early studies of ME virus no lesions were observed in any tissue other than the central nervous system. After it had been demonstrated that a relationship existed between ME and EMC viruses the pathological material was re-examined but no cardiac lesions were found. A return was made to early-passage mouse virus and the hearts of sick mice were passed intraperitoneally. Heart passage was continued for several transfers and histological studies were made of the hearts of sick animals. In animals in which the incubation period was prolonged to about 1 week or more there was evidence of myocardial lesions. Small yellow pin-point areas were seen in the myocardium on naked eye examination. Sections of these hearts showed areas of degeneration and infiltration. The degenerative areas varied in size and occasionally individual fibres would show a hyaline change.

Subsequently examination was made of animals inoculated intraperitoneally with small doses (10 to 50 LD_{50}) of early-passage mouse-brain virus and again myocardial lesions were observed in animals which had a long incubation period or prolonged paralysis. Similar lesions were observed in hamsters. In none of the primate material have any myocardial lesions been found.

EMC virus. A very detailed study of the myocardial lesions observed with this virus have been made by Schmidt (66) and by Warren (44). Apart from the myocardial lesions in the chimpanzee from which EMC virus was originally isolated all the studies have been based on rodent material. Focal areas of necrosis in the myocardium, visible as pale yellow plaques 0.5-2.0 mm. in diameter. Microscopically the extent of the lesion varied from involvement of a singel muscle fibre to an acute interstitial myocarditis involving a large area of the myocardium.

Col SK and MM viruses. Myocardial lesions similar to those found with ME and EMC viruses have been reported by Sanz Ibanez (67) in rodents infected with Col SK and MM viruses.

Lesions in chick embryos

ME virus. Chick embryos harvested 72 hours after inoculation with lethal doses of ME virus were very congested and haemorrhagic. Microscopic examination showed widespread degenerative changes, haemorrhages and thromboses. There were no specific lesions in the central nervous system or other tissues. In chick embryos harvested during the period of virus

multiplication, widespread degenerative changes were observed in the skin, musculature and visceral organs.

The chorio-allantoic membranes of embryonated eggs inoculated by the chorio-allantoic route were opaque and oedematous. On section proliferative and degenerative changes were present.

EMC, Col SK and MM viruses. Haemorrhagic and non-specific degenerative changes are recorded in embryos inoculated with these viruses (44, 60, 62).

DISCUSSION

From the evidence which has been presented above, it may be concluded that there is a family or group of viruses consisting of Mengo encephalomyelitis, Encephalomyocarditis, Columbia SK and MM viruses.

The agents of this group are not only closely related, as shown by serological tests such as have been presented above, but also by cross immunity, complement fixation, and haemagglutination inhibition tests.

Owing to the marked resistance to chemicals of the agents in this group, considerable difficulties were experienced in inactivating the viruses for the preparation of a vaccine. Thus, 20 per cent virus suspensions in as much as 0.8 per cent formolin in saline solution did not completely inactivate the agents of this group when the mixtures were allowed to stand for 48 hours at room temperature and subsequently for 14 days in a refrigerator at 4° to 6° C. In view of these findings, Warren (64) decided to vaccinate hamsters with

gradually increasing doses of the live viruses. Rodents vaccinated in this manner were subsequently challenged by intracerebral inoculation and the results of these experiments showed that EMC, ME, Col. SK and MM viruses were closely related immunologically. Confirmatory experiments by means of compliment fixation tests were also made by Warren, et al (64).

Of particular interest are the recent confirmatory studies of Olitsky and Yager (65). These investigators have brought forth evidence to indicate that the four agents in the ME family agglutinate sheep red cells. Olitsky et al concluded that it was possible by cross inhibition tests to show that ME, EMC, Col. SK and MM viruses were similar in many respects and to confirm that they were of the same group.

There is, therefore, ample evidence that this group of viruses are closely related antigenically. The relationship of the members of this group is so close that it would be reasonable to assume that they are all strains of the same infectious agent.

The evidence presented has shown that ME, EMC, Col SK and MM viruses are not serologically related to rodent-adapted or other types of poliomyelitis virus. All the rodent-adapted strains of poliomyelitis which have been tested are immunologically closely related to the Lansing-1938 strain. No relationship between ME or Col SK virus and the Lansing strain of poliomyelitis could be demonstrated. The claim of Jungeblut et al (11, 49) that Col SK virus and the related MM

virus are strains of poliomyelitis cannot be substantiated. This finding has also recently been confirmed by Powell and Jamieson (61), who showed by mouse protection tests that the Col SK and MM viruses are not immunologically related to the Lansing-1938 strain of poliomyelitis virus. The differences between this group of viruses and the strains of poliomyelitis virus are, however, not only immunological, but are also based on the host range and clinical and histopathological manifestations.

The host range of all members of the ME group is similar and includes mice, cotton rats, guinea pigs, hamsters, rabbits and monkeys. Furthermore, the agents of this group are all readily cultivated in the chick embryo. From the evidence which has been presented above in detail it may be seen that the pathogenicity of the members of this group is similar in the various hosts which have been tested. Minor strain differences have been recorded, but it would appear that these are due to the difference in the passage virus employed and to the interpretation of the investigators.

While highly neuronotropic, the ME group of agents have also marked viscerotropic characters. Thus the isolation of EMC virus from the viscera of a chimpanzee, and of one of the Mengo strains from a febrile rhesus monkey, is in contrast to the original isolation of ME virus from the CNS of a paralysed rhesus monkey. Viraemia occurs in all hosts tested prior to signs of central nervous system invasion. The primary site of virus multiplication of the Mengo group of agents is

unknown, but a rising titre of circulating virus is demonstrable for several days in the blood stream of inoculated monkeys.

In mice, after intranasal inoculation the titre of virus in the blood stream rose rapidly and reached a peak in 4 to 6 hours. No virus could be demonstrated in the brains of such mice until about the time the virus attained its maximum concentration in the blood stream. This suggests that primary multiplication of virus may take place at a site outside the central nervous system prior to its invasion of that system. Once the central nervous system has been invaded, multiplication of virus seems to be limited to that system. At the time of maximum virus concentration in the brain and cord, virus can be demonstrated in the lungs, liver, spleen, kidney, heart and blood of mice, but the presence of virus in the visceral organs could probably be accounted for by that present in the blood.

Of 18 rhesus monkeys inoculated with ME virus, only 2 showed paresis. Both of these animals had been inoculated with monkey passage virus. It is uncertain, however, if the source of the virus or the age of the monkey is of more importance in the production of paralysis in these animals. Nor is it known whether monkeys showing no signs of central nervous system involvement have nevertheless invasion of the central nervous system by virus. These problems are being studied at the time of writing.

EMC virus is said to produce a disease in monkeys which varies from a febrile illness to a paralytic infection.

In the studies of Col SK virus, Jungeblut et al (11) in 1940 stated that theyhad never observed the occurrence of frank paralysis in a single monkey which had received large amounts of Col SK mouse-brain virus by repeated intracerebral, intravenous or subcutaneous injection. The results of the experiments with ME virus have been similar to those earlier studies of Col SK virus, and more recent studies by Schultz and White (45). In 1942, however, Jungeblut et al (49) reported that after transmission through 70 mouse passages Col SK gave signs of more definite activity in the CNS of infected monkeys. No increased virulence for monkeys has been found with late mouse-passage ME virus. In general it may be said that the incidence of paralysis in rhesus monkeys inoculated with one or other of the ME group of agents is low.

In African grey monkeys (C. aethiops centralis) there is also evidence of virus multiplication after inoculation. This species is highly susceptible to extraneural inoculations with the Mengo strain (No studies in this species with other strains have been made). After a few days of fever there is usually a rapid onset of prostration and death. A similar series of events occurs in the "sooty" mangabey (Cercocebus fulginosus) after intracerebral inoculation of virus. A limited experience with Cercopithecus sabeus monkeys indicates that they react somewhat similarly to rhesus monkeys on exposure to this agent. In all tested monkeys which survived the infection, circulating antibody was demonstrated after about the end of the 1st week, and was present in high titre

by the 14th day.

Monkeys inoculated with strains of poliomyelitis behave very differently from those inoculated with the Mengo group of viruses. Fever, tremor and spasticity of muscles usually followed by paralysis are common signs. Viraemia has never been recorded in inoculated rhesus monkeys. There is, therefor, in monkeys, no clinical similarity between ME, EMC, Col S and MM virus and the virus of poliomyelitis.

Paralysis has seldom been observed in guinea pigs inoculated with the ME or the EMC strains. These animals develop an acute febrile illness with viraemia and it would appear that paralysis is more common in younger animals and when guinea pig-passage virus is employed as the inoculum. Jungeblut et al (46) established a fixed cavian strain of the Col SK virus which was characterised by the occurrence of a flaccid paralysis after intracerebral or intravenous inoculation.

Hamsters and rabbits are susceptible to inoculation with the ME, EMC and MM strains of virus both by intracerebral and peripheral routes, and like other hosts, have circulating virus in the blood stream during the acute stage of the infection. Rabbits are said to resist infection with Col SK virus. It is however, probable that with this agent (as with the other members of the group) rabbits do develop infection, not as evidenced by clinical signs but by the development of circulating antibody in their sera.

Of more importance, however, than the laboratory hosts

of these agents are the possible natural hosts. The first natural host to be demonstrated with any of these agents was the mongoose from which a strain of ME virus was isolated. In addition, 1 of 4 mongoose sera tested contained antibody to ME virus. This finding led to a consideration of other possible natural hosts. After it had been demonstrated that ME, EMC, Col SK and MM viruses were similar, a careful evaluation of the data on the isolation of Col SK virus was made. Jungeblut and Sanders (11, 49) stated that cotton rats seemed to be able to carry Col SK virus in a state of complete latency or subclinical infection. More recently, Powell et al (68) have demonstrated that the Col SK and MM viruses may be transmitted to rats and that the infected animals exhibited no symptoms but had large amounts of virus in their brains. This ability of the rat to have a subclinical infection suggests that the rats which Jungeblut et al were working with in their studies of the original poliomyelitis SK virus may have been infected with the Col SK agent which was thus accidently picked up and thought to be a variant of the original SK virus. In reviewing the geneology of Col SK virus, this seems a most likely event. Of further interest in this respect is the fact that the cotton rats emplcyed by Jungeblut et al in the Col SK isolation were Sigmodon hispidon litoralis. This species have as their habitat the eastern part of the peninsula of Florida, from Lake Harney to the Everglades (Bailey) (69). Dania (Florida) where the EMC strain was isolated (10) lies

within that area.

It is speculative to suggest that the agent was brought from Africa to Florida (by chimpanzees) and that subsequently the rat population became infected. One of these infected rats may thus have carried the virus to New York and here it was picked up by Jungeblut et al during their poliomyelitis experiments.

In view of that suggestion, it was decided to carry out some immunity studies among rats in America and in Africa where viruses of this group had been isolated and in Manila where antibody had been found in man. The studies in Africa and Manila are not yet under way but some interesting results have been obtained by Warren et al (70) in America. Eighteen per cent of a series of 442 rat sera collected in the south-eastern states of the Union were found to have antibody to EMC virus and also to ME and MM viruses. The neutralization indices were between 1,000 and 100,000. The distribution of immune rats varied considerably from region to region with the highest rate (87 per cent) occurring in rats from the state of Mississippi.

So far as other natural hosts are concerned it is unlikely from the present available data that wild arboreal monkeys carry the agent of ME. No antibody has been demonstrated in sera of wild arboreal monkeys shot in Uganda. Antibody was demonstrated, however, in 8 of 23 rhesus monkeys and 1 of 4 C. aethiops centralis monkeys caged at ground level at Entebbe.

The isolation of strains of ME virus from Taeniorhynchus

(C) fuscopennatus Theobald, and from a mixed batch of Taeniorhynchus spp. is by itself only suggestive that T. (C) fuscopennatus is a vector of ME virus. However, such a view is encouraged by the isolation of virus and demonstration of antibody in monkeys caged at ground level and in mongooses. There is experimental data showing that contact infections do not occur in monkeys. The circumstantial evidence that T. (C) fuscopennatus is a vector is strengthened by the fact that the mosquitoes from which ME virus was isolated are essentially ground-level biters. Transmission experiments have not yet been made but the available evidence is highly suggestive that the Mengo group of agents belong to the arthropod-borne virus encephalitides.

Like other members of the arthropod-borne encephalitides, all the agents of the ME group are readily cultivated in the chick embryo. In general, it may be said from the data presented that the distribution of the Mengo group of agents in the embryonated chick is similar to that of western, eastern (71) and Venezuelan (72) equine encephalitis, which agents, like ME virus, have been studied in some detail in the chick embryo.

From all the available studies, it can be stated that the lesions induced by ME virus are of a nature similar to those induced by other acute viral encephalitides. The ability of ME, Col SK, MM and EMC viruses to produce lesions in the anterior horn cells of the spinal cord is also exhibited by such viruses as yellow fever, dengue and vesicular stomatitis (73) and also (depending on the age of the animal and route of

infection) by other members of the encephalitic group, such as Japanese B, St. Louis, Russian (spring-summer), Western and Eastern equine encephalitis.

In monkeys the lesions in the cord are not unlike those of poliomyelitis. The lumbar portion of the cord appears to be the site of maximum concentration of virus and of severity of lesions. The lesions in the brain stem and brain proper are, however, quite unlike those of poliomyelitis with regard to their distribution. They are similar to the lesions induced by the equine encephalitides with the exception of the lesions in the white matter, which have been described. A complete study of the neuropathology of ME virus in primates is being undertaken at the time of writing.

Much stress has been made of the myocardial lesions induced by the virus of EMC. Myocardial lesions have been demonstrated in rodents infected with ME and with Col SK and MM (67) viruses. It must be emphasised, however, that just as anterior horn lesions in mice do not characterise any of the encephalitic viruses, so myocarditis is not a specific characteristic lesion of the Mengo group. Thus, myocarditis has been reported in fatal human cases of poliomyelitis (74, 75), the Landry-Guillain-Barré syndrome (76), influenza A (77), measles (78), mumps (79), typhus (80) and Rocky Mountain spotted fever (81); in cows, pigs and goats (82) and guinea pigs (83) inoculated with the virus of foot and mouth disease and in chickens (84) and pigeons (85) inoculated with the virus of eastern equine encephalomyelitis. It has been recorded clinically in many other virus infections (86).

It would appear that too much emphasis has been placed on the presence of myocardial lesions by Warren et al, which has resulted in the somewhat unfortunate name encephalomyocarditis, for the agent isolated by Helwig and Schmidt (10).

The size of the agents of this group are of the same order as that of poliomyelitis virus and they are similar to poliomyelitis virus and other small viruses in their marked physico-chemical stability.

This group of agents assumes importance since it has been demonstrated that man may become infected. In Uganda the Mengo strain was isolated from a human case of encephalitis and antibody to this virus was demonstrated in 3 of 233 human sera tested. 232 of these sera were obtained for yellow fever studies and no studies have as yet been made with the convalescent sera from cases of encephalitis, with the exception of the case described in this thesis. In Malaya, antibody to the EMC virus was demonstrated in 17 of 44 sera collected from patients with "3 day fever", an illness associated with encephalitic symptoms. Recently in America 9 of 200 sera examined (87) had significant levels of antibody the titre of which when tested against the EMC and ME strains were not significantly different.

The clinical syndrome is one of short duration with fever and encephalitic symptoms with, as far as is know, complete recovery. From the studies of the ME strain, viraemia occurs at some period of the febrile illness in man. In the only

case studied, virus was isolated from the blood stream on the 2nd and 3rd days of illness. Circulating antibody was demonstrable by the 10th day and was present in high titre 3 years later.

Little is known of the epidemiology of the ME, EMC, Col SK, and MM group of agents. They are certainly distributed in Africa, North America and Manila. At present, these agents are apparently capable of producing subclinical infections in their rodent hosts, but may cause a severe infection in man. Changed environmental conditions, with alteration of host-parasite relationship, could conceivably make this group, (which is perhaps at the moment of academic interest), of vital importance to human epidemiology. In this connection it may be remembered that the first epidemic of eastern equine encephalitis in man was not described till 5 years after the original isolation and study of that agent.

The study of ME virus has not only introduced much new information on the nature of the agents of this group, but has also clarified the taxonomy of this family and has clearly introduced a new etiological agent producing encephalitis in man.

Table 1

Results of cross-neutralization test with UR 22 monkey strain, T. fuscopennatus strain and Taeniorhynchus spp. strains of virus.

Virus strain	Rabbit Serum	Reciprocal of Log of LD_{50} †	Log units neutralized
UR 22 monkey	Normal UR 22 monkey strain (169 preinoculation)	6.4	-
	Immune UR 22 monkey strain (169 convalescent)	<3.0	3.4 or>
	Immune T. fuscopennatus strain (170 convalescent)	<3.0	3.4 or>
	Immune Taeniorhynchus spp. strain (171 convalescent)	<3.0	3.4 or>
T. fuscoppennatus	Normal T. fuscopennatus strain (170 preinoculation)	6.4	-
	Immune T. fuscopennatus strain (170 convalescent)	<2.0	4.4 or>
	Immune UR 22 monkey strain (169 convalescent)	<2.0	4.4 or>
	Immune Taeniorhynchus spp. strain (171 convalescent)	<2.0	4.4 or>
Taeniorhynchus spp.	Normal Taeniorhynchus spp. strain (171 preinoculation)	6.4	-
	Immune Taeniorhynchus spp. strain (171 convalescent)	<2.0	4.4 or>
	Immune UR 22 monkey strain (169 convalescent)	<2.0	4.4 or>
	Immune T. fuscopennatus strain (170 convalescent)	<2.0	4.4 or>

† All estimations of the LD_{50} in this and subsequent tables are calculated by the method of Reed and Muench (31).

Table 2

Neutralization test with acute-phase and convalescent human sera and with normal and immune monkey sera and the human and UR 22 monkey strain of ME virus.

Virus	Serum	Mortality ratio* of mice inoculated with serum plus virus dilution							Reciprocal of log of LD_{50}	Log units neutralized
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
Human	Inactivated 2nd day acute-phase	-	6/6	6/6	6/6	6/6	-	-	6.5	-
	convalescent	4/6	6/6	-	-	-	-	-	2.4	4.1
UR 22 monkey	Inactivated 2nd day acute-phase	-	6/6	6/6	4/6	4/6	3/6	0/6	6.4	-
	convalescent	1/6	1/6	0/6	0/6	0/6	-	-	2.0 or <	4.4 or >
	3rd day acute-phase	-	-	-	6/6	6/6	-	-	5.5 or >	-
	convalescent	-	1/6	0/6	0/6	0/6	-	-	3.0 or <	2.5 or >
	Normal monkey (629)	6/6	6/6	6/6	6/6	4/6	0/6	0/6	6.4	-
	immune monkey (621)	0/6	0/6	0/6	0/6	-	-	-	2.0 or <	4.4 or >

* Numerator number of mice which died.

Denominator number of inoculated mice.

Table 3

Comparison of titration obtained with ME virus is mixed with anti-sera of some of the neurotropic viruses isolated in Uganda or with yellow fever immune serum.

Serum	Source	Reciprocal of Log of LD_{50}	Log Units Neutra-lized
Normal	Rhesus 691	6.8	-
Semliki forest virus immune	Rhesus 251	6.5	0.3
Bunyamwera virus immune	Rabbit 2C	7.1	-
Ntaya virus immune	Rhesus 282	6.5	0.3
Bwamba fever virus immune	C. aethiops 668	7.0	-
Uganda S virus immune	Rhesus 922	6.4	0.4
Yellow fever immune	Rhesus 265	6.6	0.2
MEV immune	Rhesus 692	2.0	4.8 or

Table 4

Neutralization tests with ME virus and the anti-sera of some neurotropic viruses.

Serum	Source	Mortality Ratios of Mice Inoculated with serum plus virus dilution							Reciprocal of Log of LD_{50}	Log units neutralized
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}		
Normal	Rh621	6/6	6/6	6/6	6/6	6/6	6/6	0/6	6.5	-
Immune										
WEE (a)	Rh4275†		6/6	6/6					3.5 or >	3.0 or <
EEE (b)	Rh4274		6/6	6/6					3.5 or >	3.0 or <
St L. (c)	Rh4287		6/6	6/6					3.5 or >	3.0 or <
Jap. B (d)	Rh4273		6/6	6/6					3.5 or >	3.0 or <
West Nile	Rh4269		6/6	6/6					3.5 or >	3.0 or <
ME	Rh621	5/5	6/6	5/5					1.0 or <	5.0 or >
ME*	Rh722	5/6	6/6	5/5					1.0 or <	5.0 or >
Normal	Rh431		6/6	6/6	6/6	6/6	5/6	0/6	6.4	-
Immune										
Louping ill	Sh$^{\varphi}$11		0/6	0/6	1/6				4.4 or >	2.0 or <
LCM (e)	Ra273		0/6	0/6	0/6				4.5 or >	1.9 or <
ICheus	Rh4965		0/6	0/6	0/6				4.5 or >	1.9 or <
VEE (f)	Ra314		0/6	0/6	0/6				4.5 or >	1.9 or <
MR766 (g)	Rh766		0/6	0/5	0/6				4.5 or >	1.9 or <
ME	Human		3/6	6/6	6/6				2.0 or <	4.4 or >
Normal	Rh690		6/6	6/6	6/6	6/6	6/6	4/6	7.3 or >	-
Immune										
Far East (h)	Ra346				0/6	1/6	2/6		6.1 or >	1.2 or <
ME	Rh621		6/6	6/6	6/6				1.5 or <	5.8 or >

† Rhesus φ sheep * Rabbit

a Western equine encephalitis c St. Louis encephalitis e hymphocytic chorismeningitis
b Eastern equine encephalitis d Japanese B encephalitis f Venezuelan encephalitis
g. A recently isolated neurotropic virus (53) h. Far eastern (Russian) encephalitis

* A naturally immune monkey

Table 5

Neutralization tests with ME virus and anti-sera to various strains of poliomyelitis virus.

Serum	Source	Mortality ratio of mice inoculated with serum plus virus dilution								Reciprocal of log of LD_{50}	Log units neutralized
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
ME normal	Rh*4811 (preinoc.)	6/6	6/6	6/6	6/6	5/6	4/6	3/6	0/6	6.5	-
ME immune	Rh*4811 (convalescent)	0/6	0/6	-	-	-	-	-	-	<1.0	4.5 or>
Brunhilde immune	Rh B479	-	-	6/6	6/6	6/6	6/6	-	-	6.5 or>	0
Yale-SK immune	Cynomolgus 3627	6/6	6/6	6/6	6/6	6/6	6/6	-	-	6.5 or>	0
Lansing immune	Rh.C. 991, 997 & 999	-	-	-	6/6	6/6	6/6	-	-	6.5 or>	0
N.Y.City - 1944 immune	Rh 3758	-	6/6	6/6	6/6	6/6	6/6	-	-	6.5 or>	0
ME normal	Rh. pool	-	-	-	-	6/6	6/6	4/6	1/6	7.4	-
ME immune	Rh 4811 (convalescent)	-	0/6	0/6	-	-	-	-	-	<2.0	5.4 or>
MEF-1 immune	Rh 3696	-	-	-	-	6/6	6/6	6/6	-	7.5 or>	0
ME normal	Rh 431	-	6/6	6/6	6/6	6/6	5/6	1/6	0/6	6.4	
Polio. convalescent	Human pool	-	6/6	6/6	6/6	6/6	-	-	-	5.5 or>	0.9 or<
ME immune	Human	-	3/6	0/6	0/6	-	-	-	-	2.0 or>	3.5 or

* Rhesus

Table 6

Neutralization test with ME virus and antisera to the TO, GD VII and PA strains of the encephalomyelitis virus of mice

Serum	Source	Mortality ratio of mice inoculated with serum plus virus dilution.							Reciprocal of log of LD_{50}	Log units neutralized
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
TO immune	Convalescent mice pool	-	-	-	4/6	6/6	5/6	-	7.4 or>	0
GD VII immune	Rabbit No. 2	-	-	-	6/6	5/6	3/6	-	6.9	0.5
FA immune	Rabbit	6/6	6/6	-	-	6/6	-	-	6.5 or>	0.9 or<
ME immune	Rh. 4811	0/6	-	-	-	-	-	-	<2.0	5.4 or>
Normal	Rh. pool	-	-	-	6/6	6/6	4/6	1/6	7.4	-

Table 7

Cross-neutralization tests with ME, EMC, MM and Col. SK viruses and their homologous and heterologous antisera

Virus	Serum	Mortality ratio of mice inoculated with serum plus virus dilution. 10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	Reciprocal of Log of LD_{50}	Log units neutralized
ME	ME Immune 4811	0/6	0/6	0/6						
	Normal 4811				6/6	6/6	5/6		<2.0 7.4 or>	>5.4
	EMC Immune 4781	0/6	0/6	0/6						
	Normal 4781				6/6	6/6	4/6		<2.0 7.3 or>	>5.3
	MM Immune 4795	0/6	0/6	0/6						
	Normal 4795				6/6	5/6	5/6		<2.0 7.3 or>	>5.3
	Col.SK Immune 4789	0/6	0/6	0/6						
	Normal 4789				6/6	6/6	6/6		<2.0 7.5 or>	>5.5
EMC	EMC Immune 4781	0/6	0/6	0/6						
	Normal 4781				6/6	2/6	2/5		<2.0 6.0	>4.0
	ME Immune 4811	0/6	0/6	0/6						
	Normal 4811				6/6	5/6	0/6		<2.0 6.4	>4.4
	MM Immune 4795	1/6	0/6	0/6						
	Normal 4795				7/7	0/6	1/6		<2.0 5.6	>3.6
	Col. SK Immune 4789	2/6	0/6	0/6						
	Normal 4789				5/5	2/6	0/6		<2.0 5.8	>3.8
MM	MM Immune 4795	0/6	0/6	0/6						
	Normal 4795				5/6	3/6	2/6	0/6	<2.0 6.2	>4.2
	Col.SK Immune 4789	0/6	0/6	0/6						
	Normal 4789				6/6	5/6	2/6	0/6	<2.0 6.6	>4.6
	ME Immune 4811	0/6	0/6	0/6						
	Normal 4811				6/6	3/6	1/6	0/6	<2.0 6.2	>4.2
	EMC Immune 4781	0/6	0/6	0/6						
	Normal 4781				6/6	5/6	5/6	0/6	<2.0 7.3	>5.3
Col.SK	Col.SK Immune 4789	0/6	0/6	0/6						
	Normal 4789				6/6	5/6	0/6	0/6	<2.0 6.4	>4.4
	ME Immune 4811	0/6	0/6	0/6						
	Normal 4811				6/6	3/6	0/6	0/6	<2.0 6.0	>4.0
	EMC Immune 4781	0/6	0/6	0/6						
	Normal 4781				6/6	0/6	0/6	0/6	<2.0 5.5	>3.5
	MM Immune 4795	0/6	0/6	0/6						
	Normal 4795				6/6	3/6	0/6	0/6	<2.0 6.0	>4.0

Table 8

Neutralization tests with ME virus and antisera to Col. SK, MM and Columbia strains of Yale-SK and Aycock viruses and with antisera to various strains of poliomyelitis virus

Serum	Source	Mortality ratio of mice inoculated with serum plus virus dilution.								Reciprocal of Log of LD_{50}	Log units neutralized
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
ME normal	Rh.*4811 (preinoc.)	6/6	6/6	6/6	6/6	5/6	4/6	3/6	0/6	6.5	-
ME immune	Rh. 4811 (convalescent)	0/6	0/6							<1.0	5.5 or>
Col. SK immune	Rh. AQ 97		0/6		0/6		0/4			<2.0	4.5 or>
MM immune	Rabbit		0/6		0/6		0/5			<2.0	4.5 or>
Columbia strain Yale-SK immune	Rh. AR 8	2/6		0/6	0/6		0/6			<1.0	5.5 or>
Columbia Aycock virus immune	Rhesus		1/6		0/6	0/6	0/6			<2.0	4.5 or>
<u>Poliomyelitis strains</u>											
Brunhilde II immune	Rhesus			6/6	6/6	6/6	6/6			6.5 or>	0
Yale-SK immune	Cynomologous 3627	6/6	6/6	6/6	6/6	6/6	6/6			6.5 or>	0
Lansing immune	Rh. C. 991, 997 & 999				6/6	6/6	6/6			6.5 or>	0
New York City-1944 immune	Rh. 3758		6/6	6/6	6/6	6/6	6/6			6.5 or>	0

Table 9

Neutralization tests with Lansing and Yale-SK viruses and antisera to ME and Col. SK viruses

Virus	Serum	Source	Mortality ratio of mice inoculated with serum plus virus dilution.				Reciprocal of Log of LD_{50}	Log units neutralized
			10^{-1}	10^{-2}	10^{-3}	10^{-4}		
Lansing (AV2)	Normal	Rh. 4795	5/10	5/10	3/10	2/10	2.00	0.00
	Immune	Rh. pool MCPS	1/10	0/10	0/10		<1.00	>1.00
	ME immune	Rh. 4811	8/10	7/10	3/10	0/10	2.36	0.00
	Col. SK immune	Rh. 4789	9/10	10/10	9/10	0/10	3.39	0.00
Yale-SK (Yale M9)	Normal	Rh. 4795	10/10	10/10	8/10	1/10	3.43	0.00
	Immune	Cyno. 3627	1/10	0/10	-	-	<1.00	>2.44
	ME immune	Rh. 4811	8/10	7/7	9/10	6/10	3.63	0.00
	Col. SK immune	Rh. 4789	10/10	10/10	7/9	6/10	>4.00	0.00

Table 10

Comparison of average survival times in the first 10 passages of mice inoculated with human, rhesus, mongoose, mixed Taeniorhynchus, and T. fuscopennatus strains of virus.

Number of passages in mice	Average survival time in days of mice inoculated intracerebrally with 10 per cent Seitz-filtered mouse brain suspension of strain from					
	Serum			Mosquitoes		Spinal cord
	Human	Mongoose	Febrilo rhesus 823	Taenoirhynchus fuscopennatus	Taeniorhynchus spp.	Paralysed Rhesus UR 22 ✎
2	1.2	1	1.4	5	-3 survivors	5
3	1.4	1	*	6.8	-5 survivors †	3.5
4	1.0	1	*	4.4	-2 survivors †	3.5
5	1.4	*	*	3.8	3 *	3.0
6	1.2	*	*	4.6	4.2	4.3
7	1.0	*	*	2.2	2.6	2.8
8	1.4	*	*	2.6	1.4	2.2
9	1.0	*	*	*	1.5	1.6
10	1.0	*	*	*	*	2.0

✎ Line of virus isolated from a suspension of lower thoracic and lumbar cord.

* Transfer made by intraperitoneal route.

† Transfer made with 10 per cent, Seitz-filtered liver suspension.

Table 11

Comparison of titrations in mice by various routes of inoculation of 3rd and 93rd passage mouse-brain virus

Passage number	Route of inoculation	Volume inoculated, ml.	Reciprocal of Log of virus dilution	Mortality ratio	Reciprocal of Log of LD_{50}
3	Intracerebral	0.03	5 6 7	6/6 5/6 2/6	6.6
93	Intracerebral	0.03	6 7 8	6/6 6/6 6/6	8.5 or >
3	Intraperitoneal	0.06	5 6 7	6/6 6/6 4/6	7.3 or >
93	Intraperitoneal	0.06	5 6 7 8	6/6 5/6 4/6 1/6	7.2
93	Subcutaneous	0.06	6 7 8	6/6 6/6 3/6	8.0 or >
93	Intranasal	0.03	3 4 5 6 7 8	6/6 5/6 3/6 2/6 1/6 0/6	5.3

Table 12

Results of intracerebral titrations of tissues of mice inoculated with ME virus showing distribution of virus 48 hours after intraperitoneal inoculation

Tissue	Reciprocal of LD_{50}
Brain	8.3 or >
Spinal cord	8.5 or >
Spleen	4.4
Kidney	3.4
Liver	2.2
Heart	3.6
Lung	3.1

Table 13

Results of titrations of virus content in the blood and brains of mice inoculated intranasally

Expt. No.	Material tested	Reciprocal of LD_{50} at hours, ½	1	2	3	4	6	8	11	24	48	72
1	Plasma	1.0	1.5 or>	-†	1.5 or>	1.5 or>	-	1.5 or>	1.5 or>	1.5 or>	1.5 or>	1.5 or>
	Brain	0	0	-	0	1.4	-	2.4	2.0	3.6	6.5	6.3
	Serum	1.3	0	1.0	-	4.4 or>	4.3 or>	-	-	3.4	2.8	-
	Brain	0	0	0	-	0	1.6	-	-	3.8	6.4	-

† Indicates no test made.

Table 14

Data of inoculations and titres of virus circulating in blood stream of monkeys inoculated with ME virus as demonstrated by the intracerebral inoculation of mice.

Monkey		Route of inoculation	Inoculum, intracerebral LD_{50}	Source of inoculum, mouse passage	Reciprocal of LD_{50} of virus in blood on days							
Species	No.				1	2	3	4	5	6	7	8
Rhesus	621	i.c.*	8,325,000	10th	0	>1	>1	>1	>1	0	0	0
Rhesus	764	s.c.& (starch i.c.)	334	7th	0	>1	3.5	4.5	3.0	1	0	<1
Rhesus	826	i.v.†	334	7th	>2.4	>3.2	4.7	4.6	>4.2	<1	0	0
C. aethiops	529	i.c.	50,100	2nd	<1.0	2.5	3.5	3.2	1.8	<1	<1	0
C. aethiops	732	s.c. (starch i.c.)	1,112	38th	0	<1	1.6	3.0	5.0	5.3	2.0	Died day 7
C. aethiops	627	i.v.	1,112	38th	<1.0	2.3	3.5	5.5	6.3	Sacrificed Day 5		
C. aethiops	626	s.c. & i.m.‡	70,000,000	18th	>1.0	>1.0	>1.0	>1.0	>1.0	Died Day 6		

* i.c. intracerebral

& s.c. subcutaneous

† i.v. intravenous

‡ i.m. intramuscular

Table 15

Distribution of virus in the central nervous system of C. aethiops inoculated with MEV compared with that of a naturally infected paralysed rhesus monkey

Suspension of	Mortality ratios of mice inoculated intracerebrally with Seitz-filtered suspensions of portions of central nervous system of			
	C. aethiops			Rhesus
	732	627	626	UR 22
Cortex	3/6	-†	1/6	0/6
Medulla and pons	0/6	-	2/6	0/6
Cerebellum	0/6	-	2/6	5/5
Midbrain	0/6	6/6	0/6	1/6
Cervical cord	1/6	-	4/6	3/6
Thoracic cord	2/6	-	6/6	-
Lumbo-sacral cord	6/6	-	6/6	6/6*

† Indicates no test made.

* Included portion of thoracic cord

Table 16

Titres of MEV in saline and serum-saline suspensions stored at incubator temperature

Time stored (days)	Reciprocal of LD_{50} titre of virus stored at incubator temperature (37.5°C.)	
	Saline suspension	Serum-saline suspension
0	6.6	6.6
7	4.3	5.6
13	2.9	3.4
25	1.0	1.0 or >
33	- †	1.0

† No test made.

Table 16

Titres of MEV in saline and serum-saline suspensions stored at incubator temperature

Time stored (days)	Reciprocal of LD_{50} titre of virus stored at incubator temperature (37.5°C.)	
	Saline suspension	Serum-saline suspension
0	6.6	6.6
7	4.3	5.6
13	2.9	3.4
25	1.0	1.0 or >
33	- †	1.0

† No test made.

Table 17

Comparison of thermostability of ME virus when Seitz-filtered 10 per cent mouse-brain virus is heated with 10 per cent serum or with a serum-free diluent.

Diluent employed	Mortality ratio of mice inoculated with virus suspensions heated for 30 minutes at °C.		
	60	70	80
10 per cent monkey serum-saline	6/8	4/8	8/16
0.1 per cent bovine albumin-saline	0/8	0/6	0/16

Table 18

Results of tests of the effects of various chemicals on the viability of Mengo encephalomyelitis virus.

Chemical	Final percentage concentration	Mortality ratio in mice inoculated after contact of					
		Minutes			Hours		
		12	30	60	6	24	72
Phenol	1	2/6	0/6	1/6	2/6	0/6	0/6
Formaline	1	6/6	5/6	5/6	0/6	0/6	-
	0.5	6/6	6/6	4/6	0/6	0/6	-
Alcohol	1	6/6	6/6	6/6	6/6	6/6	6/6
	5	6/6	6/6	6/6	6/6	6/6	6/6
Ether	50	6/6	6/6	6/6	6/6	6/6	5/5
Hydrogen peroxide*	1	6/6	6/6	6/6	6/6	4/6†	0/6
Potassium permanganate	0.5	0/6	0/6	0/6	0/6	0/6	-
	0.05	3/6	1/6	1/6	0/6	0/6	-

† H_2O_2 titrated after 24 hours and found to be approximately 0.9 per cent.

Table 19

Virus content of chick embryo harvested 24, 48 and 72 hours after amniotic inoculation

Time, Hours.	Mortality ratio of mice inoculated with virus at dilution							Reciprocal of mouse LD_{50}
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
24	5/5	6/6	2/6	0/6	0/6	0/6	0/6	3.7
48	6/6	6/6	6/6	6/6	6/6	3/6	0/5	7.0
72	6/6	6/6	6/6	6/6	6/6	5/6	1/6	7.5
4	0/5	0/5	0/4	0/5	0/6	-	-	<1.5
21	5/5	4/5	2/5	1/5	0/5	-	-	3.8
48	-	-	-	5/5	5/5	5/5	1/5	7.6
96	-	-	-	5/5	5/5	5/5	-	7.5

Table 20

Distribution of virus in various parts of the embryonated egg after inoculation by the amniotic, yolk sac allantoic and chorio-allantoic routes

Tissue tested for virus	Route of inoculation	Reciprocal of LD_{50} of virus at hours after inoculation	
		24	48
Embryo	Amniotic	3.8	7.63
	Yolk sac	4.5	5.9
	Ch. Allantoic	1.5	4.8
	Allantoic	1.5	1.5
Yolk sac	Ammotic	-†	6.3 or
	Yolk sac	-	6.3 or
	Ch. Allantoic	-	4.8
	Allantoic	-	1.5
Allantoic fluid	Amniotic	1.5	-
	Yolk sac	1.5	-
	Ch. Allantoic	1.5	-
	Allantoic	1.5	1.7

† No test made.

FIGURE 1

TEMPERATURE CHART OF RHESUS 739 INOCULATED DIRECTLY WITH SERUM OF HUMAN CASE.

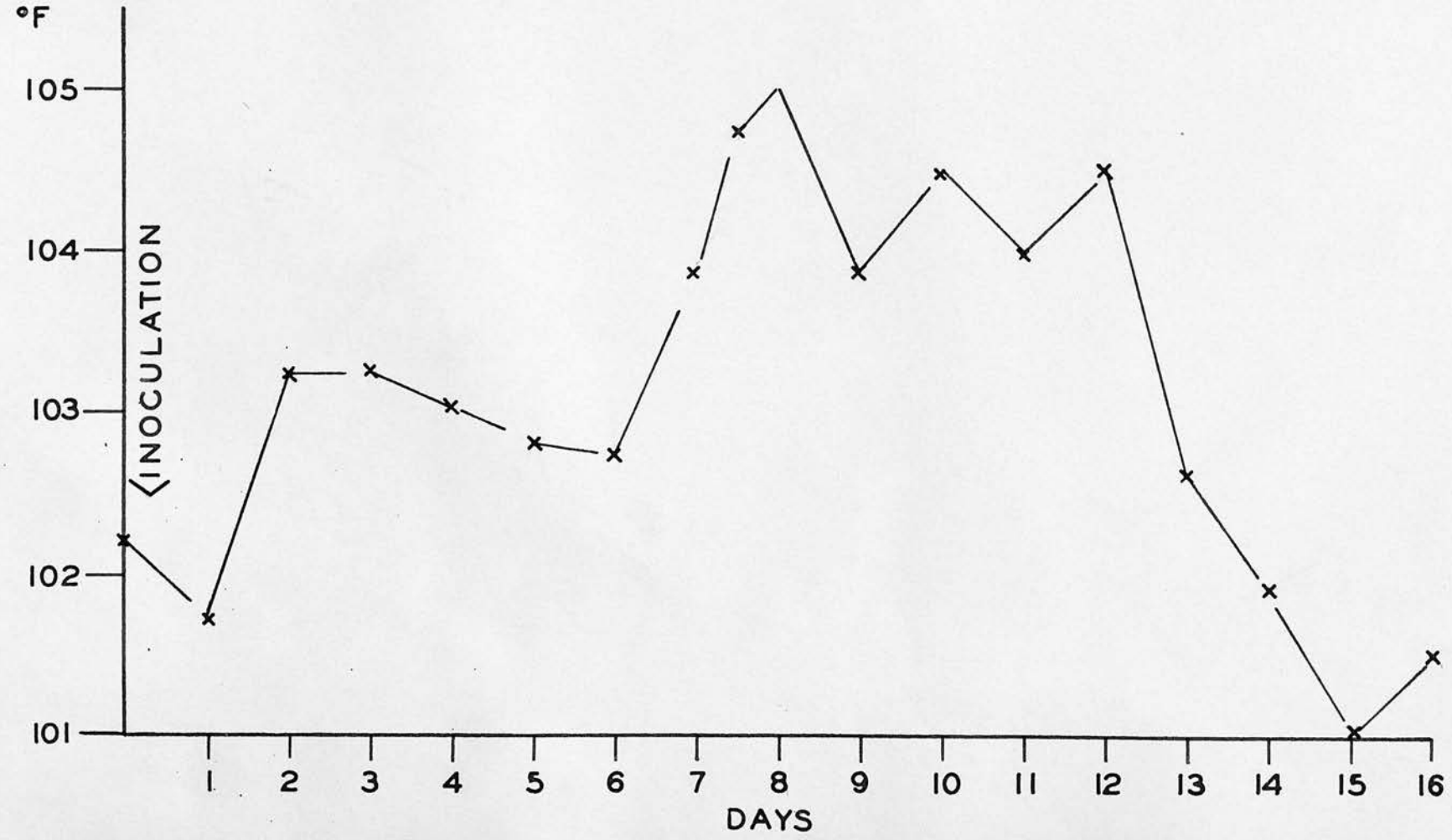


FIGURE 2

TEMPERATURE CHART OF RHESUS 600 INOCULATED WITH 8TH DAY SERUM OF RHESUS 739.

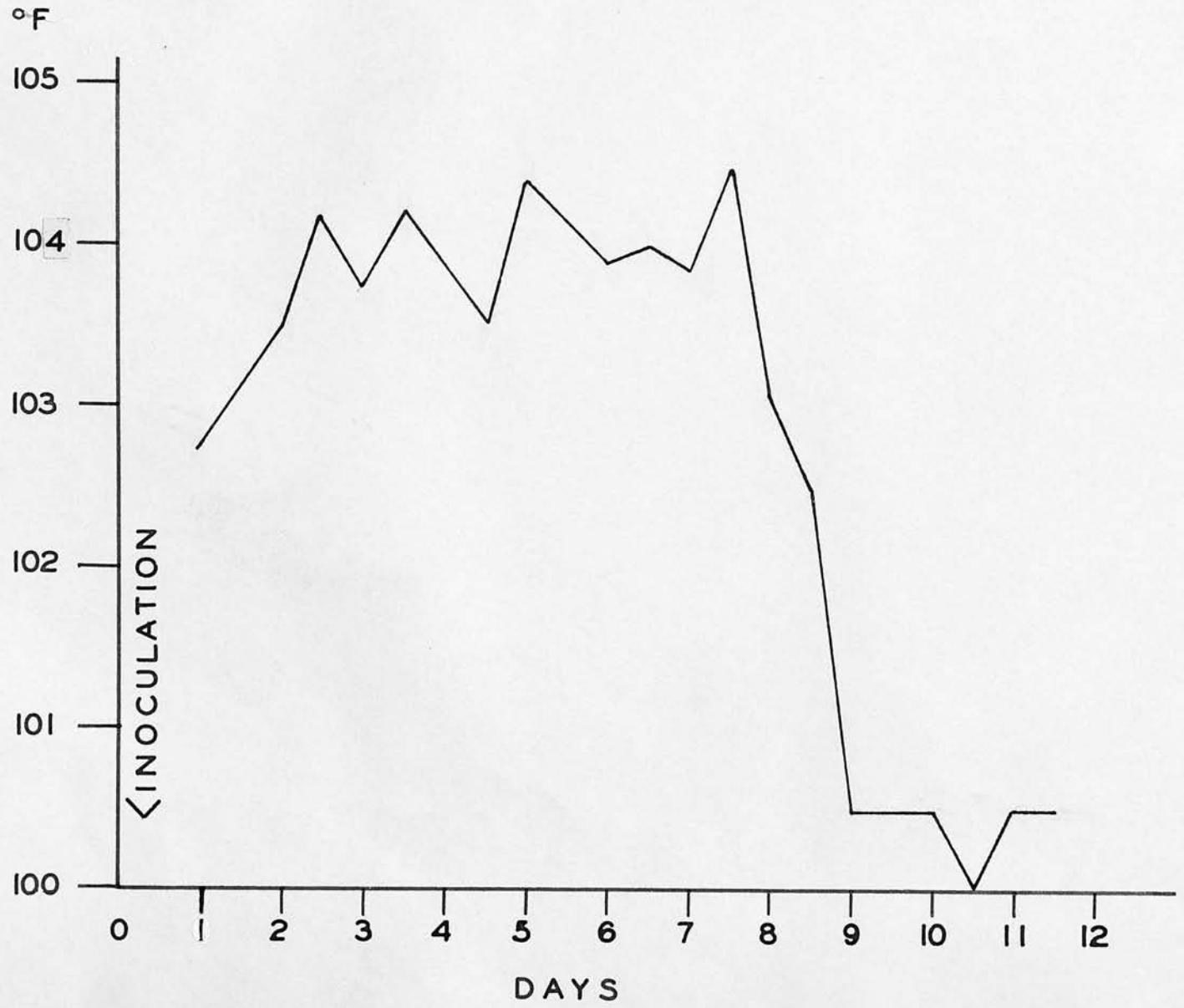


FIGURE 3

TEMPERATURE CHART OF HUMAN INFECTION WITH ME VIRUS

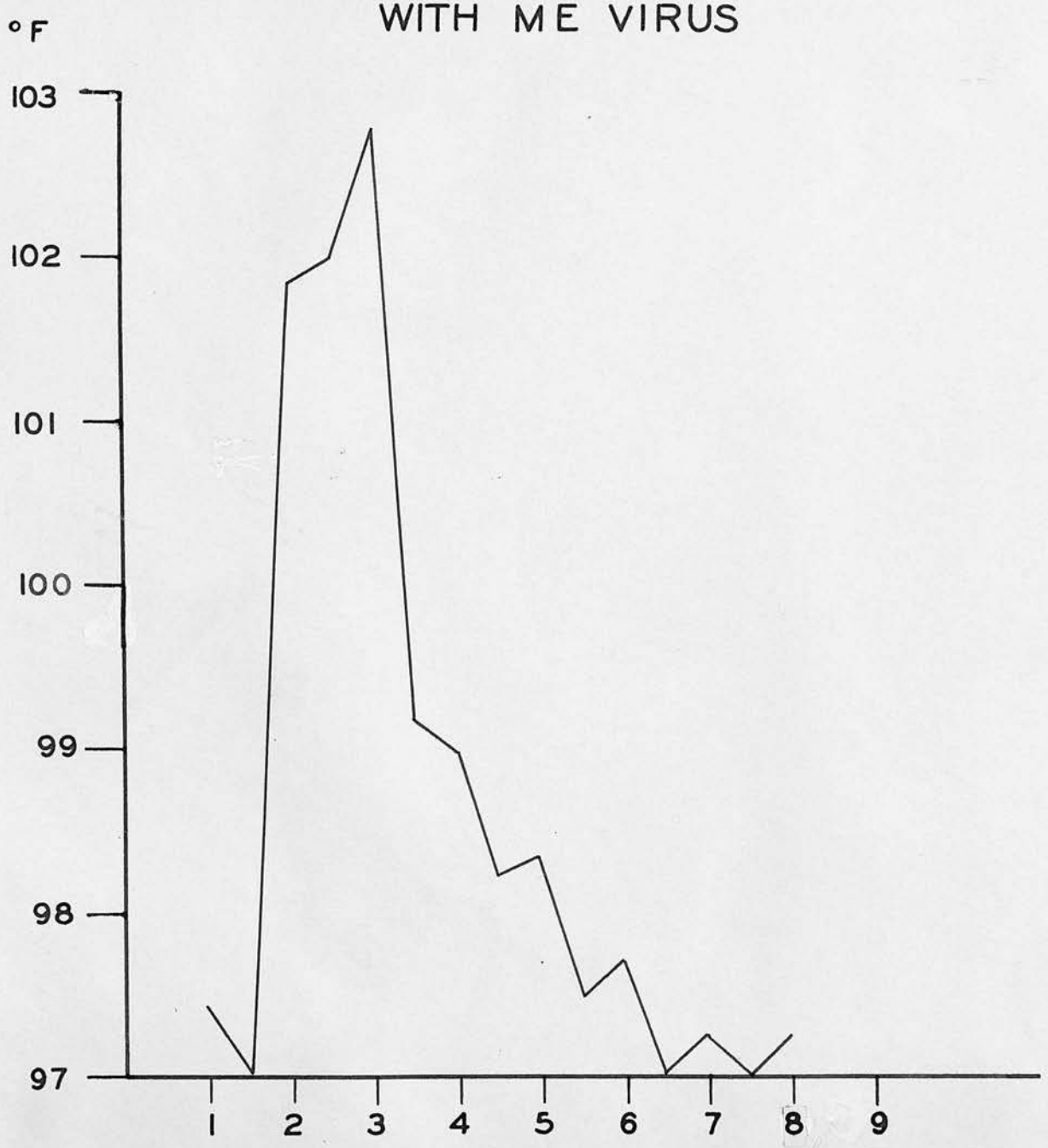
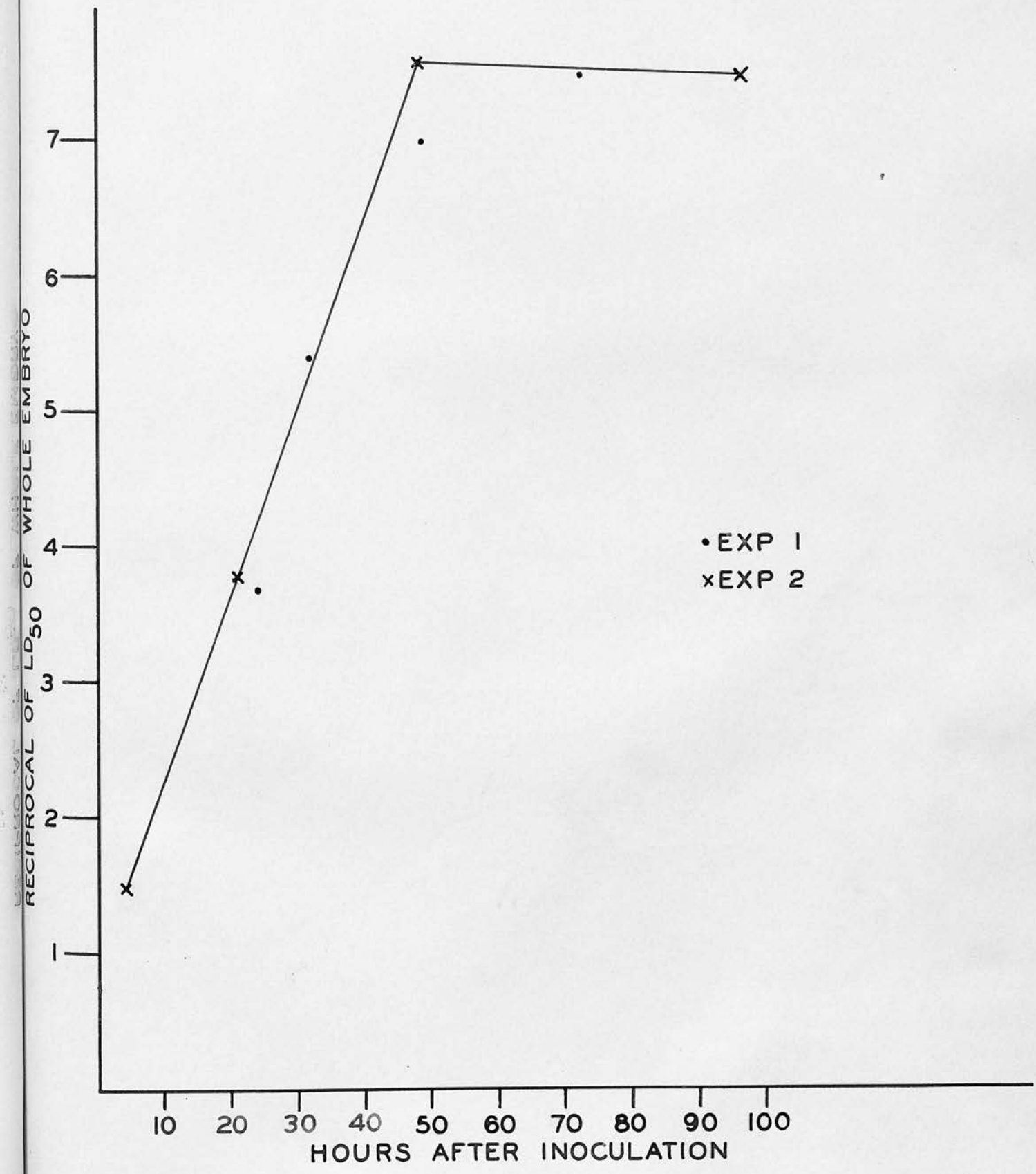


FIGURE 4 -

RATE OF MULTIPLICATION OF ME VIRUS IN CHICK EMBRYO AFTER AMNIOTIC INOCULATION



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APPENDIX I

BIOGRAPHICAL NOTE

G. W. A. Dick.

The candidate was born in Glasgow, Scotland on August 14, 1914. He was educated at Hutchinson's Grammar School, Glasgow and the Royal High School, Edinburgh, from where he went to the University of Edinburgh. He graduated M.B.Ch.B. in 1938 and B.Sc. (1st Class Hons. Pathology) in 1939. He was awarded a vans Dunlop Scholorship in Physiology and the Buchanan Scholarship in Obstetries and Gynaecology during his undergraduate years. In October 1939 he was appointed resident house-physician to Professor Murray Lyon. During that year he passed the clinical examination for the degree of Doctor of Medicine. In March 1940 he was appointed Assistant Pathologist at the Royal Informary, Edinburgh, but relinquished that post the same month to join the Army.

He served for 6 years in the Royal Army Medical Corps at first as a specialist in Pathology and later commanded the Medical Division of a General Hospital with the rank of Lt. Colonel. He was on active service in Abyssinia, Somaliland, Madagascar, East-African territories, Mauritius, and North Africa.

On demobilization he was appointed as a pathologist at the Yellow Fever Research Institute, Entebbe, Uganda. In 1948 whilst on a Rockefeller Foundation Fellowship in America, he was awarded the degree of Master of Public Health by the Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland. Subsequently he was appointed as Research Fellow in Epidemiology at Johns Hopkins University to under-

take research at the Poliomyelitis Research Center.

APPENDIX II

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